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Design, Properties and Applications

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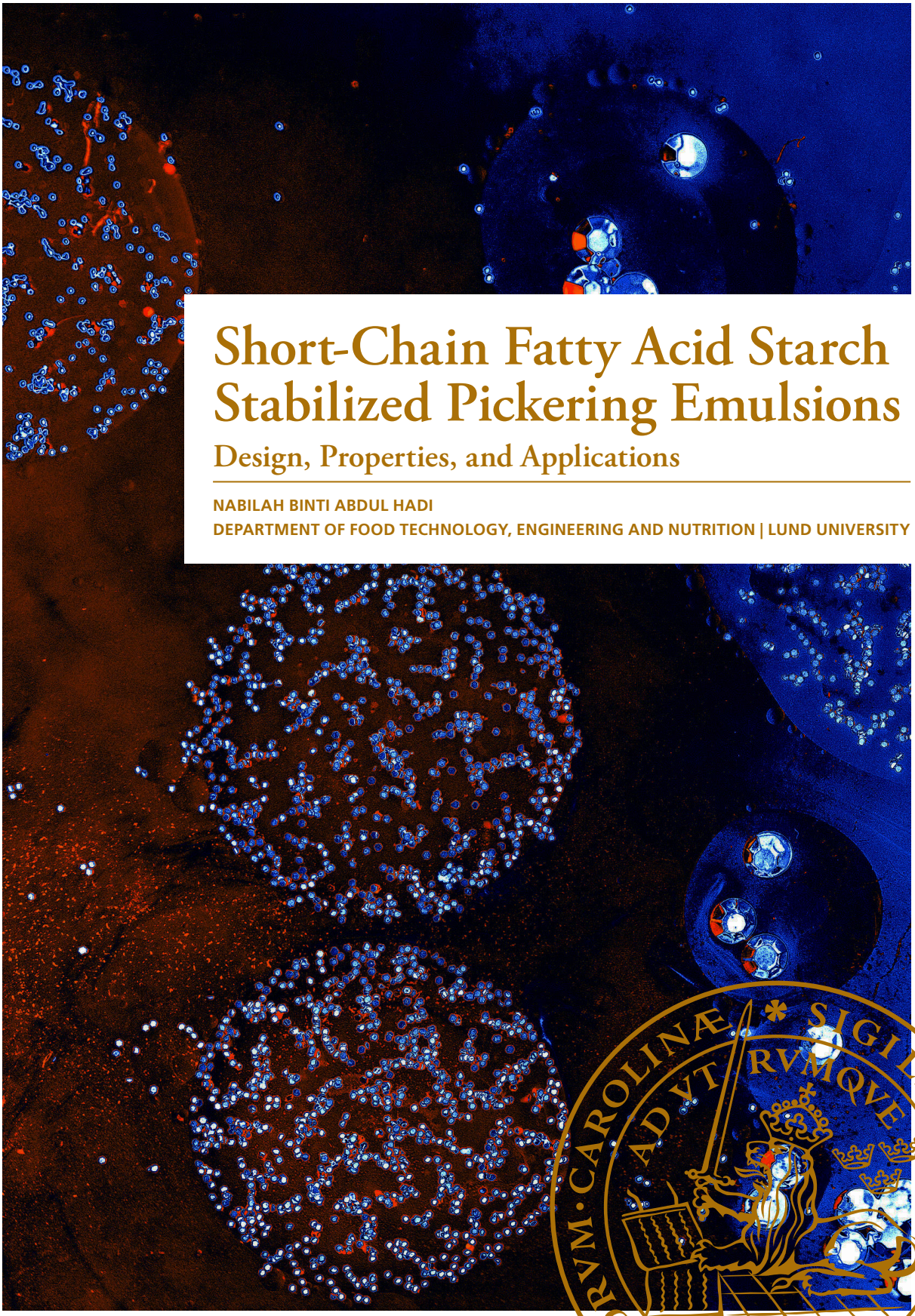

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-The Little Things Can Sometimes Be the Most Priceless-

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Lund University

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Nabilah Binti Abdul Hadi

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Pickering emulsions are emulsions stabilized by solid particles. Particles with optimal dual wettability toward both of the oil and water phases, can be adsorbed onto the interface, thereby providing the stability of the emulsions. Starch granules have attracted attention due to their positive characteristics, such as being widely available, inexpensive, biodegradable, and non-allergenic. Due to a relatively low degree of hydrophobicity, chemical modification of starch can improve starch hydrophobicity by esterification with a short-chain fatty acid (SCFA) group. The aim of this thesis was to perform SCFA starch modification by means of esterification of rice and quinoa starches with different SCFA groups and levels of modification. The physicochemical and functional properties of SCFA starches were investigated. As one of the future applications, the emulsifying capacity of SCFA starches was evaluated and in vitro digestion was carried out. Until recently, there have been no studies evaluating the effect of different types of SCFA starches and the levels of modification to the physicochemical properties, emulsification and digestibility. The rationale behind the selection of different types of SCFA starches at different levels of modification and the application of these to stabilize Pickering emulsions were discussed. The esterification of starch with short-chain fatty acids group was successfully quantified by direct stoichiometry, FTIR and ^{1}H-NMR. SCFA starches have shown a different properties compared to their native forms. Native and SCFA-rice starches have a larger particle size compared to native and SCFA-quinoa starches. Both types of starches displayed a polyhedral shape. Upon modification, no changes in particle size were observed. SCFA starches exhibited a reduction in protein and amylose content. SCFA starches demonstrated low gelatinization and pasting temperature. The highest level of resistant starch was observed in the starches with the highest level of modification. Principle component analysis revealed that the physicochemical and functional properties of SCFA starches are highly influenced by the level of modification. SCFA starches were able to perform as a stabilizer in Pickering-type emulsions. The emulsifying capacity was improved by increasing SCFA chain length and levels of modifications. SCFA-quinoa starch Pickering emulsions were observed to have smaller droplet sizes, higher emulsification index, better Turbiscan stability index, and more stable droplet sizes that remained below 50 µm during 50 days of storage. This indicated that Pickering emulsions stabilized by SCFA-quinoa starches were more stable than SCFA-rice starches. *In vitro* digestion of SCFA starch Pickering emulsions showed that increasing SCFA chain length and modification level reduced the extent of starch hydrolysis. The results of this PhD project implied that increasing the chain length and modification level improved the overall hydrophobicity of the granules and hence improves the emulsification capacity and stability. Improved hydrophobicity resulted in a higher adsorption degree (less free starch) and a denser layer of particles at the interface. Hence, this dense layer protects the oil droplets and prevents the enzyme from getting access to the oil droplets. However, particle coverage was not complete due to the large sizes of the particles. There were therefore still small gaps between starch particles, resulting in lipolysis not being completely arrested. In future research, formulation of SCFA starch Pickering emulsions can be used to investigate the capacity of these emulsions to serve as a carrier for controlled release and targeted delivery of bioactive compounds to a specific location of the gastrointestinal tract, such as the distal locations of the small intestine or the colon.

Keywords: short-chain fatty acid starch, physicochemical properties, Pickering emulsions, starch modification, emulsion stability, starch hydrolysis, *in vitro* digestion, lipolysis

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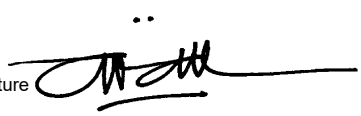
Signature  Date 22/03/2021

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“Keep your feet on the ground
and keep reaching for stars.”

Casey Kasem

Contents

Abstract

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Popular scientific summary

Emulsions are a special type of formulation created by two or more immiscible phases, such as oil and water with the aid of emulsifiers or stabilizers. Emulsions are constructed by one phase dispersed within a continuous phase in the form of small droplets. Emulsions are ubiquitous on daily basis. They are present in cosmetics (e.g. hand cream, lipsticks, and face serum), foods (e.g. ice cream, butter, milk, and mayonnaise), and pharmaceutical products (e.g. encapsulate drugs, topical medication and self-emulsifying tablets). Despite being effective as an emulsion stabilizer, emulsifiers such as synthetic surfactant could cause problems to human health and the environment, such as skin irritation and water pollution. Moreover, people are very concerned about sustainable and green-label ingredients. Thus, a selection of emulsifiers that fulfil those requirements has caused researchers to focus attention on finding healthy and environmentally-friendly emulsifiers that also provide the desired emulsion properties.

One approach is to replace the chemically-synthesized emulsifiers with natural solid particles with a similar emulsifying capacity. The emulsions developed by solid particles are called Pickering emulsions. In Pickering emulsions, solid particles are adsorbed at the interface of emulsion droplets. Those solid particles surround the emulsion droplets, creating a barrier that protects the droplets from coalescence. Compared to non-biodegradable particles, such as silica, clay and alumina particles, renewable and edible biomaterials like starch have gained interest for the stabilization of Pickering emulsions. Pickering emulsions are stable under a wide range of pH, temperature, and oil type. In addition to food, starch has been used in various applications, such as pharmaceuticals, cosmetics, and packaging. In some cases, the properties of starch are not optimal and it therefore needs to be modified. One way to overcome this is through chemical modification. Chemically-modified starches have been developed over the years to improve its emulsification properties in general and are categorized as food-grade starch, such as octenyl succinic anhydride starch (E1450) and acetylated starch (E1420). In this work, native starches from rice and quinoa were modified with three different types of short-chain fatty acids (SCFA) of different chain lengths, namely acetyl, propionyl and butyryl.

The first part of this thesis aimed to provide more details about SCFA-rice and SCFA-quinoa starch modification and verification of its degree of substitution (DS), as this is fundamental knowledge for verifying the effectiveness of the starch

modification method. In the second part of the thesis, native and SCFA starches were characterized in terms of physical, chemical and digestibility. This work is aimed at finding the relationship of types and levels of modification to starch properties, in order to be able to provide insight for industrial applications. As the third part of this thesis, SCFA starches were used to stabilize Pickering emulsions. Various aspects of Pickering emulsions were evaluated (i.e. droplet size, emulsifying capacity, stability) to determine SCFA starches' capacity to stabilize Pickering emulsions. Lastly, the final part of this thesis is to highlight the *in vitro* digestibility of SCFA starch Pickering emulsions.

The results of this thesis have demonstrated that native rice and quinoa starches were successfully modified with SCFA by verification of its degree of substitution. Esterification of the SCFA group disrupts the inert structure of the starch granules, thereby changing the physicochemical properties of starch. In addition, modification altered the digestibility of the starch in SCFA starches. The modification with the longest SCFA chain length and highest levels of modification improved the emulsification capacity of SCFA starch stabilized Pickering emulsions. This resulted in smaller droplet sizes, less free oil, and less free starch. SCFA starch Pickering emulsions were stable for 50 days of storage, while the small droplets' size was retained. The emulsifying capacity was improved due to the increase in hydrophobicity of SCFA starch particles, thereby increasing the affinity of the particles to adsorb on oil droplets. A low amount of hydrolyzed starch was obtained in SCFA starch Pickering emulsions. Starch modified with the longest SCFA chain length had high resistant (indigestible) starch content. This condition was observed in butyrylated starch Pickering emulsions. Moreover, strong adsorption of SCFA starch on the surface of oil droplets improved starch surface coverage, thus creating particle barriers surrounding the oil droplets, which limits the extent of lipid digestion.

This thesis could give comprehensive information to the industry in the design and selection of SCFA starches and SCFA starch Pickering emulsions that suit their requirements. Compared to traditional emulsions, SCFA starch Pickering emulsions are easy to produce, nontoxic, stable, and have a high emulsifying capacity. SCFA starch Pickering emulsions are valuable in providing health benefits to control blood sugar level. The approach of SCFA starch Pickering emulsions can also be utilized in food and pharmaceutical applications when encapsulation, protection, controlled release and targeted delivery of bioactive materials (e.g. vitamins, carotenoids, fatty acids, amino acids or drug compounds) to distal locations in the gastrointestinal tract is in focus.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

Paper I: **Abdul Hadi, N.**, Wiege, B., Stabenau, S., Marefati, A., & Rayner, M. (2020). Comparison of Three Methods to Determine the Degree of Substitution of Quinoa and Rice Starch Acetates, Propionates, and Butyrates: Direct Stoichiometry, FTIR, and ^{1}H-NMR. Foods, 9(1), 83.

Paper II: **Abdul Hadi, N.**, Marefati, A., Purhagen, J., Humphreys, B., Wiege, B., Nylander, T., & Rayner, M. Physicochemical and functional properties of starch modified with short-chain fatty acid at different acyl groups and levels of modification. Manuscript.

Paper III: **Abdul Hadi, N.**, Marefati, A., Matos, M., Wiege, B., & Rayner, M. (2020). Characterization and stability of short-chain fatty acids modified starch Pickering emulsions. Carbohydrate Polymers, 240, 116264.

Paper IV: **Abdul Hadi, N.**, Rayner, M., Wiege, B., & Marefati, A. *In vitro* digestion of Pickering Emulsion Stabilized by Short-Chain Fatty Acid Modified Starch Granules. Manuscript.

Additional publications not included in this thesis

Paper V: Marefati, A., Wiege, B., **Abdul Hadi, N.**, Dejmek, P., & Rayner, M. (2019). *In vitro* intestinal lipolysis of emulsions based on starch granule Pickering stabilization. Food Hydrocolloids, 95, 468-475.

Paper VI: Boostani, S., Hosseini, S. M. H., Golmakani, M.-T., Marefati, A., **Abdul Hadi, N. B.**, & Rayner, M. (2020). The influence of emulsion parameters on physical stability and rheological properties of Pickering emulsions stabilized by hordein nanoparticles. Food Hydrocolloids, 101, 105520.

Author's contribution to the papers

Paper I: Abdul Hadi, N. and Wiege, B. performed the experimental design. Abdul Hadi, N., Wiege, B. and Stabenau, S. performed SCFA starch modification and determination of the degrees of substitution (stoichiometric calculation and FTIR). Abdul Hadi, N. performed an NMR experiment. Abdul Hadi, N. and co-authors analyzed the results. Abdul Hadi, N. wrote the major part of the paper.

Paper II: Abdul Hadi, N. planned the entire experimental design. Abdul Hadi, N. and Marefati, A. planned the *in vitro* starch digestion experiment. Abdul Hadi, N. performed all of the experimental work, Abdul Hadi, N. and Humphreys, B. performed and analyzed SAXS. Abdul Hadi, N. and co-authors interpreted the results. Abdul Hadi, N. wrote the paper.

Paper III: Abdul Hadi, N. and co-authors planned the experimental design. Abdul Hadi, N. performed all of the experimental work. Abdul Hadi, N. and co-authors analyzed the results. Abdul Hadi, N. wrote the paper.

Paper IV: Abdul Hadi, N. together with Marefati, A. designed the *in vitro* digestion of starch stabilized Pickering emulsions. Abdul Hadi, N. performed all of the experimental work. Abdul Hadi, N. and co-authors analyzed the data. Abdul Hadi, N. wrote the paper.

Aim and objectives

The aim of this thesis is to design short-chain fatty acid-modified starch granules that can be used to stabilize Pickering emulsions. A deep understanding of modified starch was first acquired, followed by their capacity to stabilize Pickering emulsions. Lastly, the functional properties of SCFA starch stabilized Pickering emulsions were determined.

This overall aim was divided into the following specific objectives, which were divided into the following papers:

Paper I:

- To modify starch with a short-chain fatty acid with different types and levels of modification
- To verify the degree of substitution of short-chain fatty acid-starches through different types of analysis techniques

Paper II:

- To investigate physicochemical and functional properties of SCFA starches and establish the relationship between types and levels of modifications of those properties.

Paper III:

- To formulate SCFA starch stabilized Pickering emulsions with respect to different starch concentration, types and level of modifications
- To evaluate the stability of SCFA starch Pickering emulsions

Paper IV:

- To examine the digestibility of SCFA starch Pickering emulsions *in vitro* and the influence of type of modification on starch hydrolysis and lipolysis.

Background

Emulsions

Emulsions are defined as mixtures of at least two immiscible liquids in phases, whereby one phase is dispersed in the other phases in the form of droplets [1, 2]. A dispersed phase or internal phase is referred to as a constituent that forms droplets in an emulsion system; whereas a continuous phase or external phase is the surrounding liquid in which droplets are suspended. An emulsion is categorized into two types depending on their spatial distribution of the oil or water phases. A system made up of oil dispersed in a water phase is called an oil-in-water (O/W) emulsion, with examples such as mayonnaise, cream and milk. Conversely, if water is dispersed in an oil phase, the system is defined as water-in-oil (W/O) emulsion, with examples such as butter and margarine [1]. In the food context, emulsion droplet diameters commonly range from 100 nm to 100 μm. However, droplet sizes also can reach 5–50 nm (micellar emulsions or micro-emulsions) [1, 3].

It is possible to mix two immiscible liquids using a device that provides intense mechanical energy, such as high-shear mixers or high-pressure homogenizers. However, the droplets formed in this way tend to come together rapidly and coalesce. This results in separation of the oil and aqueous phases, whereby the phase with the lower density (usually oil) is at the top and the other phase (usually water) is at the bottom due to density differences dictated by the gravitational effects. This is a result of the fact that emulsions are thermodynamically unstable systems. To prevent this physicochemical mechanism, a stabilizer, like an emulsifier or texture modifier (thickener and gelling agent) is necessary to improve their kinetic stability and prevent coalescence for a longer shelf life [4]. Emulsifiers, such as surfactants and biopolymers, are surface-active molecules that can attach at the interface of droplets. This process is described as adsorption (Figure 1). The adsorption of emulsifiers thereby reduces the surface and interfacial tension between oil and water in an emulsion system based on the nature of the emulsifiers [5] (Table 1). Lowering the interfacial tension allows droplets to be broken up to a greater extent in the laminar and turbulent flow during emulsification, causing a reduction in droplet size [6]. Other stabilizers, for example, texture modifiers stabilize an emulsion by improving the viscosity of the continuous phase, thereby hindering droplet movements. The selection of stabilizers also demonstrates different stability

mechanisms to stabilize emulsions. Emulsion stabilization by electrostatic repulsion is generated by the adsorption of an ionic surfactant that forms a charged layer at the interface. A double layer is then created by the counterions, which creates charged droplets. Each of the charged droplets is repelled due to strong repulsive forces, thus preventing coalescence [7, 8]. Conversely, stabilization via steric hindrance commonly forms by nonionic surfactants, macromolecules or solid particles. These types of emulsifiers create a physical barrier between the oil and water interface to prevent coalescence [1]. The interfacial layer thickness (monolayer) for droplets stabilized by food emulsifiers, such as surfactants, phospholipids and proteins, is in a range of 1–10 nm. The interfacial layer might be thicker or have multilayers depending on the types of emulsions [9]. Therefore, apart from being used as food ingredients or finished products, emulsions have been recognized as attaining good performance as a delivery vehicle in functional foods for bioactive components like fatty acids, antioxidants, vitamins and carotenoids [10, 11].

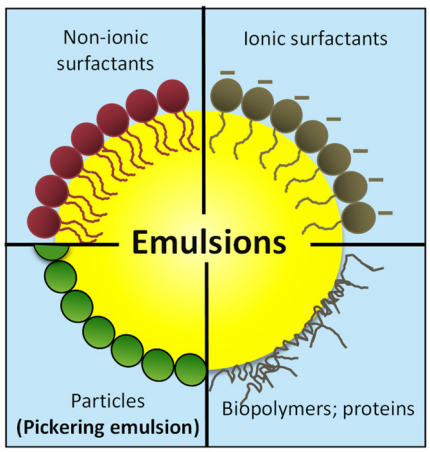

Figure 1. Schematic representation of oil-in-water emulsion stabilized with surfactants, biopolymer, and particle-stabilized emulsions.

Table 1. Comparison based on characteristics and functional properties of emulsifiers that are commonly used as food emulsifiers.

Emulsifier Class	**Small Molecular Weight Surfactants**		**Macromolecules**		**Particles**	
Size	~ 0.4 to 1 nm		2-200 nm		10 nm to 10 μm	
Chemical class	Non-ionic	Ionic	Amphiphilic biopolymers		Colloidal solids $\theta < 90^{o}$	Colloidal solids $\theta > 90^{o}$
Examples	Polysorbates, monoglycerides	Phospholipids, Sodium stearoyl lactylate	Proteins; Egg/ dairy proteins	Polysaccharides; Gum Arabic	Solid particles; modified starch and cellulose	Fat crystals
Surface active	Yes		Yes		Yes	
Amphiphilic	Yes (hydrophilic "head" group and lipophilic "tail" group)		Yes (hydrophobic and hydrophilic regions)		No (unless Janus particles)	
Desorption energy	< 10kT		several thousand kT		> several thousand kT	
Solubility	Water or oil	Water	Water	Water	Water	Oil
Emulsion type	O/W or W/O	O/W	O/W	O/W	O/W	W/O
Molecular organization in solution	Micelles, bilayers, vesicles, and reverse micelles		Globular, random coil, or rod-like		Particles or particles aggregates in dispersion	
pH stability	Good	Good	Poor at IEP	Good	Good	Good
Salt stability	Good	Poor at I > CFC	Poor at I > CFC	Good	Good	Good
Temperature stability	-	Poor at T ~ PIT	Poor at T > T_m	Good	Good	-

Note: IEP (isoelectric point), PIT (phase inversion temperature), T (temperature), Tm (thermal denaturation temperature), I (ionic strength) and CFC (critical flocculation concentration).
Source: Dejmek et al., [8] and McClements [1].

Pickering emulsions

Pickering emulsions are emulsions that use solid particles as emulsifier to stabilize two immiscible phases. The difference between traditional and Pickering emulsions is that, once the particles are adsorbed on the droplet interface (oil or water), energy of adsorption is high owing to the large sizes (usually > 10 nm). As a result, the energy of detachment is so high that this adsorption is considered irreversible. A mechanical barrier is formed in Pickering emulsions through the formation of a thick layer of solid particles [12-14]. This special mechanism prevents Pickering emulsions against coalescence and Ostwald ripening (Dickinson, 2012).

Pickering emulsions stabilized by starch and protein particles have a considerably higher droplet size (>10 μm), which can be affected by the size of particles used to stabilize these droplets (sub-micron to micron-sized particles) [15-18]. The adsorption of particles is dependent on their wettability, with the contact angle of 90º being the best when the particles are equally wetted by both phases (particle is not too hydrophilic or too hydrophobic) (Figure 2). In the case of O/W Pickering emulsions, the contact angle is less than 90º, which means that the particles are wetted more by the water phase (hydrophilic particle). Conversely, when the contact angle is greater than 90º, W/O is formed where particles are wetted more by the oil phase (hydrophobic particle). Without dual wettability, particles would not be able to attain maximum stabilization on Pickering emulsions, which could result in particle displacement by other materials present in an emulsion system [19-21]

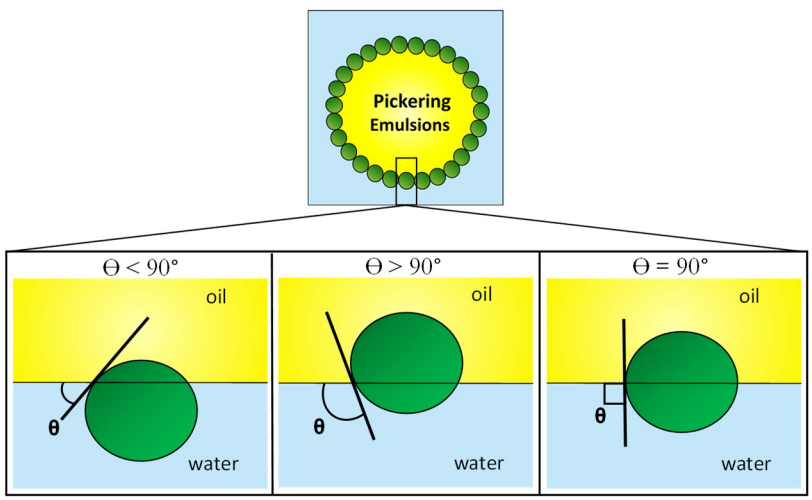

Figure 2. Schematic of particle location on a water-oil interface, contact angle (θ) is measured through the water phase.

According to Binks et al., [22], the location of adsorbed particles on the oil-water interface alters the interfacial tension of oil and water and thereby the free energy of an emulsion system. By assuming the adsorbed particle is a sphere and the effect of gravity is negligible due to the small particle sizes, the free energy or energy of detachment (ΔG) required to desorb a particle of radius (r) from an oil-water interface is defined by the following equation [20]:

$$\Delta G = \pi r^2 \gamma \, (1 - \cos \theta)^2 \qquad (1)$$

where γ is the interfacial tension between the oil and water, θ is the contact angle between the particle and water, r is the particle radius.

The energy of detachment can reach up to 10^4 kT (particle radius ~ 10 nm) if the θ is 90°, denoting that it is difficult to remove particles from the water-oil interface. This indicates that the emulsion system reaches maximum stabilization with an irreversible state. Conversely, the energy of detachment is low when the contact angle is extremely high $\theta > 160°$ (hydrophobic), or too low $\theta < 20°$ (hydrophilic). This facilitates desorption of a particle from the oil-water interface [20-23]. Therefore, in a Pickering emulsion system without the presence of surface-active agents, the wettability of particles on an oil-water interface is solely dependent on the affinity of the particles towards the water or oil phase. Particles that are too hydrophilic or small are not desirable for stabilization Pickering emulsions.

The formulation of a Pickering emulsion consists of solid particles, a continuous phase, a disperse phase, and the presence of energy to create an emulsion. Examples of continuous phases are water and phosphate buffer, while short, medium or long-chain triglycerides may act as disperse phase [24-27]. Factors affecting the properties and stability of Pickering emulsions have previously been discussed in the literature. These include particle concentration, particle size, type of particle, oil-water ratio, and aqueous and oil type [24-26, 28-32]. Several studies have shown that increasing particle concentration in Pickering emulsions produces small droplet sizes due to the larger total surface area of the particles that is available to cover the droplets, thus forming a small droplet size. Additionally, an appropriate number of particles makes it possible to achieve a complete surface coverage (dense layer) of particles at the interface, preventing close contact between droplets [33-35]. A detailed study by Aveyard et al., [21] has described how Pickering emulsion droplet size decreases as particle concentration increases, and once particle concentration exceeds a maximum amount, the droplet sizes remain constant at this point. This is because the power of the homogenization device cannot break the drops further even though there is an excess of particles that could, in theory, stabilize any new interface formed [20]. The excess particles thus remain in a continuous phase as free particles. Additional stabilization arises by those free particles, preventing interaction between droplets and also increasing continuous phase viscosity, which can improve emulsion stability [36, 37].

Mechanism of emulsion instability

Emulsions are thermodynamically unstable systems, thus there is a tendency to separate into two distinct phases or layers over time. Emulsions are considered to be kinetically stable when no or a low detectable rate of changes are observed in an emulsion system over time. Examples of emulsion instability mechanisms are creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion (Figure 3). One notable emulsion instability phenomenon is gravitational separation, which occurs as creaming or sedimentation. Creaming and sedimentation can be identified when emulsions display the presence of a distinct cream layer at the top or bottom of the container, respectively. Creaming occurs when droplets have a lower density than that of the continuous phase, causing droplets to move upwards. Sedimentation, on the other hand, is a process whereby droplets move downwards due to having a higher density than the surrounding liquid [2]. The gravitational separation rate, v (m/s), is defined by Stokes' law as presented below:

$$v = \frac{2}{9}\frac{r_p^2\left(\rho_p - \rho_f\right) g}{\eta} \tag{2}$$

where r_p is a spherical radius of the particle, ρ_p is the density of the particle, ρ_f is the density of the dispersed phase, g is the acceleration due to gravity, and η is the viscosity of the continuous phase.

A simple approach to suppressing creaming is by minimizing the density difference between the phases, adding weighting agents to the phase with lower density, reducing the droplet radius, and increasing the viscosity of the continuous phase [38]. An emulsion can also show a flocculation process if minimum interaction energy is present, causing emulsion droplets to stick to each other [39, 40]. The flocculate droplets are separated by a thin film of the continuous phase, which means that each droplet still retains its identity. Flocculation may proceed with two mechanisms either by bridging or depletion flocculation. In contrast to flocculation, coalescence involves an irreversible merger of emulsion droplets or flocculated droplets causing the formation of larger spherical droplets. Coalescence usually occurs in an extended period or takes place after droplet aggregation [41]. Ostwald ripening occurs due to the higher Laplace pressure of small droplets compared to the large droplets, allowing the diffusion of small droplets and their material onto the bigger droplets, resulting in an increase in average emulsion droplet size [42]. The switching of an emulsion system between O/W emulsion and W/O emulsion is known as phase inversion or defined as catastrophic or transition by Dickinson [43]. For a convectional emulsion, phase inversion is induced by changes in emulsifier concentration, oil and water, temperature, degree of agitation, and electrolyte concentration that influence the hydrophile-lipophile balance (HLB) of the system

[44]. Unlike emulsions stabilized by a surfactant, phase inversion in Pickering emulsions can be induced by altering particle wettability or increasing the volume fraction of the dispersed phase. This phenomenon is accompanied by changes in the stability and size distribution of the emulsions [37].

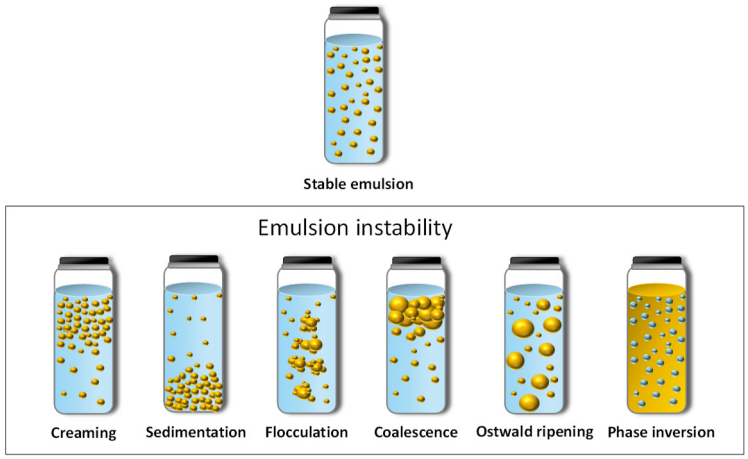

Figure 3. Schematic diagram illustrating the instability process of emulsions.

Starch

Starch structure

Among carbohydrates, starch is a polymer that is made by most of the green plants and stored in the form of granules in plant tissues and organs (e.g. seeds, roots, shoots, leaves, grains, and stems) as a source of energy [45]. Starch granules are semi-crystalline and composed of two types of polymers, amylose and amylopectin, that differ in both size and structure. Amylose is a linear chain of α-D-glucose units that are linked by α-(1-4) glycosidic bond. Amylose is located in the amorphous regions of starch granules [46]. While amylopectin is a branched molecule formed by α-(1,4)-glucan chains joined with α-(1,6)-glycosidic bonds (Figure 4). Amylopectin contributes to the crystallinity of starch through ordered arrangements of double helices formed by adjacent branches within the structure [47]. In starch granules, amylopectin is classified into three different subchains, defined as A, B and C type, which differ in length. The A-type amylopectin is the shortest chain, with a degree of polymerization (DP) of 19–28. It is mostly found in normal maize, rice and cereal starches. B-type is found in tuber starches (e.g. potato and lotus root)

with DP range 29–31. C-type, with DP 25–27, is a mixture of long and short chains of A and B-type. It is found in banana, and smooth pea [48].

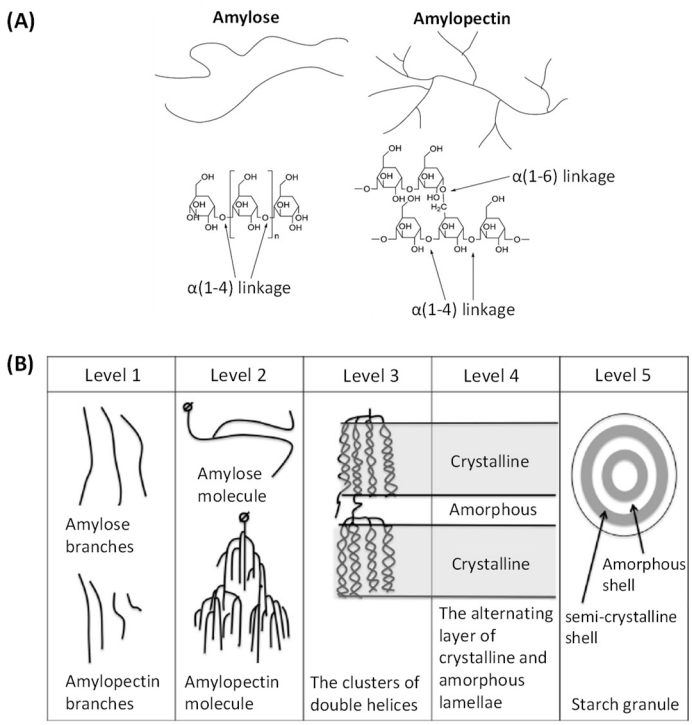

Figure 4. Isomers of starch: amylose and amylopectin (A) and schematic illustration of the hierarchical structure of starch (B) adapted from Giri et al., [49] and Wang et al., [50].

Granule size and morphology

Starch granules vary in shape, size, and composition depending on the botanical origin. Starch granules have a wide range of sizes, categorized as large granule, >25 μm (e.g. potato, rye); medium granule, 10–25 μm (e.g. tapioca, barley); small granule, 5–10 μm (e.g. rice, oat); and lastly extremely small granule, <5 μm (e.g. quinoa, amaranth) [51] (Figure 5). The size of starch granules can also be reduced from their original size to nanometers. Nanosized starch can be achieved through fractionation, ultrasonic and acid or enzymatic treatment [52-54]. In terms of the shape of starch, rice, corn and quinoa starch granules exhibit polyhedral, angular, and irregular shape [55, 56]. Starch granules from root or tuber, such as potato starch, exhibit oval-shaped granules with a smooth surface [57, 58]. Meanwhile, the shape of tapioca starch is spherical with a truncated corner [59].

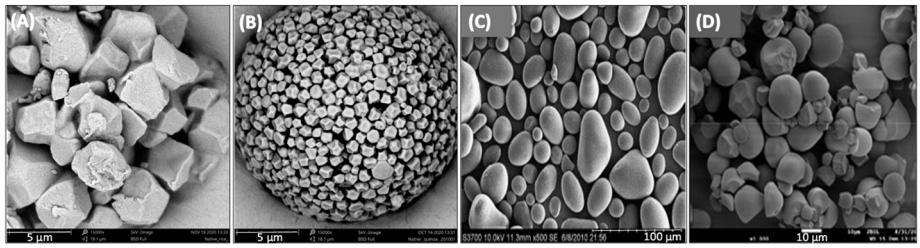

Figure 5. SEM images of native starches at different magnification; (A) rice (15000x), (B) quiona (15000x), (C) potato (500x) adapted from Zhu et al., [58], and (D) tapioca (1000x) adapted from Prompiputtanapon et al., [60].

Thermal properties

Starch is used in food applications and is thus normally exposed to processes that involve heating. Thermal transitions of starch are therefore representative information for evaluating starch functionality. The physical transformation of starch during heating can be measured through gelatinization, enthalpy, and viscosity. Gelatinization takes place when starch is heated in the presence of water. Initially, water is diffused inside starch granules with limited swelling. This results in the disappearance of birefringence, morphological changes, and the leaching of amylose. This phenomenon is also an irreversible disruption of the molecular arrangement within starch granules [61, 62]. Thermal properties of starch can be measured using Differential Scanning Calorimetry (DSC), which defines the temperature of gelatinization onset (T_O), peak (T_P), conclusion (T_C), and enthalpy (ΔH) change. The gelatinization temperature of starch depends on its botanical origin and the amylose amylopectin composition of the starch. For example, the gelatinization temperature range for some different types of starches are as follows: potato starch is from 55 to 66 °C, wheat starch from 52 to 63 °C, maize starch from 62 to 72 °C, and rice starch from 66 to 77 °C [63-65]. When starch granules reach their gelatinization temperature and are continuously heated in an excess of water, starch continuously swells to several times its original size due to the diffusion of water inside the starch granules (Figure 6). The transition during gelatinization and swelling can be investigated by measuring its pasting viscosity. The pasting viscosity can be recorded using a viscometer, such as Brabender Visco Amylograph or Rapid Visco Analyzer (RVA).

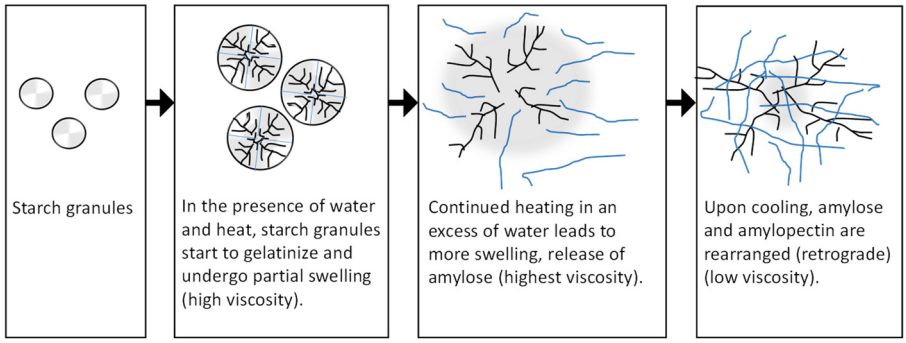

Figure 6. Schematic illustration of starch gelatinization and swelling.

The pasting viscosity is measured throughout the process from the starting point of gelatinization until the end of the cooling step. When starch starts to gelatinize, starch granules start to swell, causing an increase in viscosity as temperature increases. The peak viscosity is reached when swelled granules reach equilibrium, with amylose leaching out into solution [66]. Further stirring results in the rupture of granules, which results in a further decrease in the viscosity. Upon cooling, starch molecules reorganize to some extent to form a gel (possibly with retrogradation), in which amylose molecules aggregate into a network [61] (Figure 6).

In vitro digestibility of starch

In vitro digestion of starch provides a measure of the rate and extent of sugar release as a result of starch hydrolysis by simulating the physiological processes occurring in the gastrointestinal tract. Starch digestion involves the action of enzymes (in solution) that diffuse and bind to the starch granules as a substrate, followed by catalytic actions (cleaving the glycosidic linkages) [67]. The rate of starch hydrolysis is affected by various factors, such as structural organization (amorphous and crystalline), morphology (granule size and shape), and starch treatment (chemical modification, enzymatic modification, heat-moisture treatment and annealing) [68]. Starches from different botanical origins vary in their amylose and amylopectin fraction. Starches with high amylose content have a low extent of hydrolysis attributed to some amylose forming the double helices packing in the external region of starch granule [69]. In terms of the processing state, raw starch (depending on its source) is slowly or partly resistant to digestion compared to cooked starch, which is fully gelatinized. For example, cooked butyrylated high-amylose maize starches are less susceptible to starch hydrolysis compared to cooked native starch [70].

Investigation of *in vitro* starch digestion and the extent of hydrolysis can give rise to understanding that enables strategic modulation of starch digestion and glucose absorption. Englyst et al., [71] proposed a method for the classification of starch

into fractions based on its digestibility as a means of addressing the physiological fate of starch. According to this method, starch can be classified into three fractions based on the time needed for the enzymatic hydrolysis during *in vitro* intestinal digestion. Rapidly digestible starch (RDS) is a starch fraction that is digested within 20 min of hydrolysis. Slowly digestible starch (SDS) is the starch fraction that is digested in between 20 and 120 min. Meanwhile, resistant starch (RS) is the remaining starch fraction that is not digested within 120 min of *in vitro* intestinal digestion [71, 72].

RDS is the starch fraction that can lead to an increase in glycemic response, which causes a rapid increase in blood sugar and insulin levels that is detrimental to health [73]. SDS is digested slowly in the small intestine, thereby prolonging the release of glucose, which is considered to have a moderate glycemic response. SDS is preferable for the development of ingredients that can help to reduce blood sugar and control hyperglycemia [74]. RS is the fraction of non-digestible starch that is resistant to the hydrolytic action of α-amylase and is not absorbed in the small intestine. Thus, RS is a so-called dietary fiber that can act as the substrate for microbial fermentation in the large intestine [75, 76]. RS is potentially able to maintain the pH of the bowel at a low pH condition and result in the prevention of overgrowth of pH-sensitive pathogenic bacteria [75].

RS can be categorized into four groups; Type I, Type II, Type III, and Type IV. RS type I (RS1) is found in starchy foods that are not fractionated and refined which can be found mostly in legumes and cereals (beans and lentils). RS type II (RS2) is resistant starch granules with the B- or C- polymorph crystal structure, such as unripe banana and raw potato. RS type III (RS3) is retrograded starch, such as cooked and cooled potato and stale bread, since retrogradation occurs when starches are cooked (gelatinized) and cooled (room temperature, refrigerator, or freezer) for a period of time which can result in the formation of RS. Finally, RS type IV (RS4) is a resistant starch found in chemically-modified starches such as acetylated, cross-linked starch [72, 77].

Chemical modification of starch

Although native starch has been widely used in commercial applications, it is sometimes challenging to choose a suitable starch due to limitations associated with their physicochemical and functional properties in some particular applications. Native starch exhibits low shear stress resistance, thermal resistance, low emulsifying capacity, retrogradation and syneresis that impairs the final properties of different products [33, 78, 79]. To address these shortcomings, modification of starch before its final use is one way to attain desirable properties. Examples of starch modification are chemical modification (e.g. cross-linking, esterification, and

etherification), physical (e.g. ball milling, annealing, and shear modification), enzymatic (e.g. α- and β- amylases, transglucosidase and pullulanase) or a combination of two modification methods (e.g. acetylation/annealing, debranching/hydroxypropylation and extrusion/succinylation) [80-82].

The amylose chain of starch has a helical conformation with six anhydroglucose units per turn. The outer surface of the helix is composed of hydroxyl groups of glucosyl residues, and the hydrophobic part is located at the internal cavity. In the chemical modification of starch, particularly esterification, hydroxyl groups in the glucose units within the amylose helix are therefore substituted with an ester group [61] (Figure 7). There are three reactive hydroxyl groups in each glucose unit, making the maximum degree of substitution (DS) value three [83].

An example of starch chemical modification is esterification with octenyl succinic anhydride (OSA). OSA modification of native starch has been investigated and applied to stabilize Pickering emulsions [27, 33, 84-87]. Among esterified starches, researchers have focused in particular on propionylated and butyrylated starch due to their capacity to deliver specific SCFA to the colon. Short-chain fatty acids (SCFA) have been associated with the improvement of certain gut diseases, such as colorectal cancer, suppression of colonic inflammation, and the hindrance of an overgrowth of pathogenic microorganisms [70, 75, 88, 89]. Acetylated starch has been widely studied to improve the properties of food products [90, 91]. Meanwhile, propionylated and butyrylated starches have recently gained attention as a source of resistant starch that can potentially work in starch-based delivery systems [92, 93]. Short-chain fatty acids may therefore be an option that can be used in the chemical modification of starch. Another important thing is that the SCFA acyl groups can increase the hydrophobicity of starch, making them suitable for certain applications, including their use as emulsifiers [29, 94, 95]. Furthermore, the fabrication of starch granules by esterification with SCFA is one of the novel green chemical processes that is safe for application in relation to human health [96].

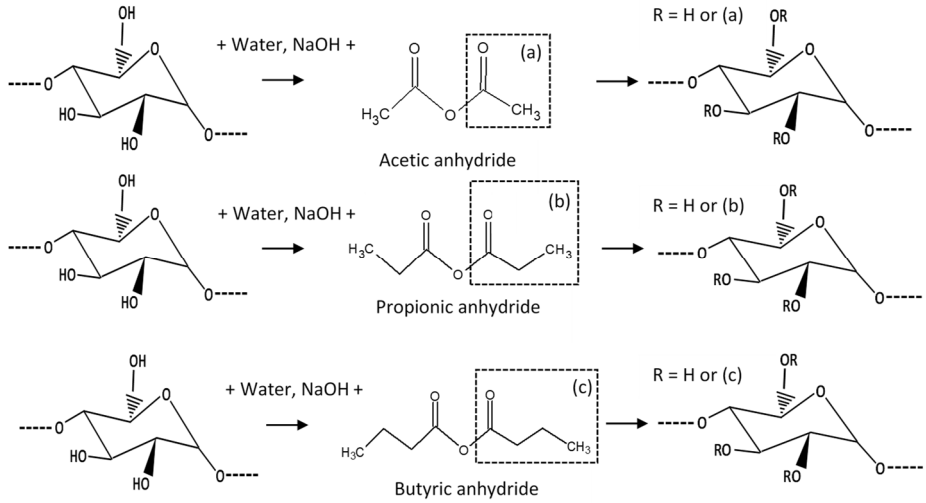

Figure 7. Schematic representation of the esterification process between acyl anhydride and starch structure under alkaline conditions.

Starch granules as Pickering emulsifiers

Depending on the types of ester group and degrees of substitution, chemical modification of starch improves its hydrophobicity [97]. As a result, modified starch can achieve desirable partial dual wettability towards the oil-water interface, thereby creating a densely-packed layer (monolayer or multilayer) around the dispersed droplets (Figure 8). This phenomenon creates a barrier that is able to hinder coalescence and flocculation of the emulsion droplets, thus improving the stability of Pickering emulsions [98]. This mechanism has been demonstrated by [20, 27, 99] using OSA-treated quinoa and waxy maize starches. Even though native starch granules can stabilize Pickering emulsions, they are prone to exhibit instability and appear as free starch dispersed in the aqueous phase due to hydrophilic surface properties and poor dual wetting capacity [28, 33, 97]. Droplet sizes of Pickering emulsions prepared using native starch granules are normally large, typically 100 μm to a few mm depending on the size of the starch granules used [100]. Large starch granules form larger emulsion droplets, which is unfavorable due to less efficient granules packing at the interface. This leads to short-term instability of emulsions due to gravitational separation, coalescence, and Ostwald ripening [30, 31, 101]. A study by Li et al., [102] concluded that the diameter of starch granules affects the stability of Pickering emulsions. Their studies used native starch granules from different botanical sources (waxy maize, wheat, potato and rice) to stabilize Pickering emulsions. Emulsions prepared with rice starch granules exhibited better emulsifying capacity and stability against coalescence during storage compared to

potato starch granules. However, the emulsifying capacity of such large particles is poor due to the large size of potato starch granules and lower available surface area for the same mass of particles. As a result, more granules are required to stabilize the droplets [103, 104]. In addition, through chemical modification, the surface character of naturally hydrophilic starch granules is improved to more hydrophobic, giving it a greater ability to adsorb at the oil-water interface. Increasing the degree of substitution of esterified starches has been shown to improve the emulsifying capacity of starch granules in the formation of Pickering emulsions [28, 33, 105].

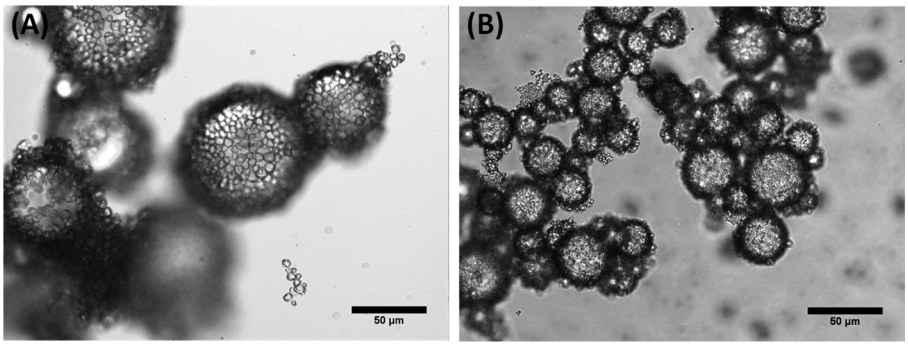

Figure 8. Light microscopy images of Pickering emulsion droplets showing that butyrylated rice (A) and butyrylated quinoa (B) starch particles covered the emulsion droplets.

In vitro gastrointestinal digestion of Pickering emulsions

In vitro digestion studies of Pickering emulsions have mostly been performed using static models adapted from Minekus et al., [106], where salivary, gastric and small intestinal digestion is mimicked in three consecutive steps [107-110]. In some cases, the oral phase of the *in vitro* digestion is excluded for liquid formulations because liquid does not remain in the mouth for more than a few seconds. However, it is useful to start with the oral phase when a starch component is present. The oral phase involves a combination of simulated salivary fluid (SSF), α-amylase and/or amyloglucosidase enzymes at neutral pH (6.8–7) [111]. In the widely-used static models of *in vitro* gastric digestion, the gastric motility, gastric secretions over time and gastric emptying rate are not included, and the sample is simply incubated in a stirred simulated gastric fluid (SGF) at 37 °C for one to two hours [112]. *In vitro* gastric digestion operates through the action of the pepsin enzyme, gastric lipase, and hydrochloric acid. In the human body, the stomach environment is generally acidic (pH 1.2–3.0). The acidic environment in the gastric phase is due to the secretion of hydrochloric acid by parietal cells, and this secretion is varies from one individual to another [113]. Simulated intestinal fluid (SIF) must be prepared at

neutral pH (6.8–7.0), with relevant enzymes (e.g. amylase for carbohydrate hydrolysis, proteases for protein hydrolysis and lipase for lipolysis) and biological surface-active components (bile salts, and phospholipids) to mimic the digestion conditions in the small intestine. Digestion in the small intestine involves a break-down into smaller fragments. For example, starches are broken down into monosaccharides, proteins are digested into amino acids or peptides, and lipids are digested into free fatty acids and monoglycerides. The by-products of digestion are transported across epithelial cells by an absorption mechanism [114].

Emulsions have been frequently proposed as a formulation approach for the delivery of bioactive compounds to the desired sites of the human body. The majority of these studies adapted a static *in vitro* digestion model, as this method can predict end-point values (free fatty acids, glycemic index, micronutrient bioaccessibility) [115-117]. The amount of free fatty acid released in Pickering emulsions upon digestion in the gastrointestinal (GI) tract can be traced by measuring the volume of sodium hydroxide used to neutralize the free fatty acids. The extent of lipolysis in Pickering emulsions can be attributed to its droplet size and stability. Previous findings have stated that small emulsion droplets are more easily hydrolyzed by lipase due to the large surface area, allowing more accessible surfaces for the lipase to act on [118]. Thus, even though an emulsion with a small droplet size is more stable than one with large droplet sizes, droplet size is not the only factor that can determine the extent of lipolysis. The physical organization of particles surrounding the oil droplet is an important matter to consider. Study from Bai et al., [119] have addressed that a high concentration of solid particles can densely cover the interface of oil droplets, thereby improving their surface coverage and acting as a barrier to prevent the transport of lipase and bile salts to the oil droplets. Since Pickering emulsions are stabilized by solid particles, they are superior in hindering lipolysis compared to conventional surfactant-based emulsifiers. This is because the particles have high desorption energy, which makes it difficult for the displacement of particles from the droplet interface by bile salts [118, 120, 121]. The lipolysis rate of Pickering emulsions is also affected by the types of oil used in the emulsion system. Very recent works highlighted that the composition of triglyceride (TAG) with saturated and unsaturated fatty acid profiles can influence the extent of lipolysis, where unsaturated and long-chain triglyceride demonstrated a low extent of lipolysis resulted due to limitation of calcium's and bile salts' ability to remove the free fatty acids from the interface of oil droplets [122]. Calcium plays a critical role in the dynamics of lipid digestion, by which calcium ions bind to free fatty acids to form an insoluble calcium soap, thereby removing free fatty acids from the droplet surface [123]. Bile salts also act in the removal of digestion by-products from the oil droplets. When the bile salt concentration is not adequate, the lipolysis end products (i.e free fatty acids, mono and diacylglycerols) accumulate at the interface of oil droplets, thereby limiting the availability site for pancreatic lipase [118, 124, 125].

General methods

Modification of starch with SCFA

Chemical modification of native rice and quinoa starches with short-chain fatty acid via esterification was performed as described in **Paper I**. Three SCFA groups with different acyl chain lengths (i.e. acetic, propionic and butyric anhydride) at different concentrations were used. The esterification was performed in an alkali condition by the addition of 0.7 M NaOH at a controlled pH of 8.5.

Degree of substitution of SCFA starch

Stoichiometric calculation

The most common method used to determine the degree of substitution of SCFA starches is titration based on the hydrolysis of the ester bonds in an alkali solution [126]. The titration method requires a large amount of sample (1 g) and is time-consuming. In this work, instead of using the titration method, three methods were proposed to compare the DS of SCFA starches. The first method was based on stoichiometric calculations. Two reactions are involved in the acylation of starch with SCFA. The main reaction is the reaction of the alkaline-activated starch St-$O^{(-)}$ with the anhydride. Meanwhile, a reaction of anhydride with $OH^{(-)}$ ions is a side reaction, as shown below:

Main reaction:

$$\mathrm{St-OH + OH^{(-)} + (CH_3CO)_2O \rightarrow St-OCOCH_3 + CH_3COO^{(-)} + H_2O} \quad (3)$$

Side reaction:

$$\mathrm{(CH_3CO)_2O + 2\ OH^{(-)} \rightarrow 2\ CH_3COO^{(-)} + H_2O} \quad (4)$$

where St-OH represents starch, $(CH_3CO)_2O$ is acetic anhydride and St-$OCOCH_3$ represents starch acetate. In the main reaction, one mole of $OH^{(-)}$ is needed for the consumption of one mole of acetic anhydride. For the side reaction, two moles of $OH^{(-)}$ are needed to neutralize the pH. The ratio of the molar amount of $OH^{(-)}$ to the molar amount of anhydride must therefore be between 1 (only main reaction) and 2

(only side reaction). By taking the reaction parameters into the account (molar amount of educts), the degree of substitution and acyl content are calculated stoichiometrically from the derived equation by considering the molar amount of educts. From the moles of anhydride (n(Anhyd.)) and sodium hydroxide (n(NaOH)) required during the esterification, the DS of SCFA starches was calculated using the following equations:

$$n(\text{Anhyd.-St.}) = 2 \times n(\text{Anhyd.}) - n(\text{NaOH}) \quad (5)$$

$$DS = \frac{n(\text{Anhyd.-St.})}{n(\text{Anhyd.Glu})} \quad (6)$$

where n(Anhyd.-St.) is the moles of anhydride that is chemically bond to the starch, n(Anhyd.Glu) is the moles of anhydroglucose. The number of moles of anhydroglucose is determined using the following equation:

$$n(\text{Anhyd.Glu}) = \frac{m(\text{Anhyd.Glu})}{M(\text{Anhyd.Glu})} \quad (7)$$

where m (Anhyd.Glu) is the mass of anhydroglucose, M(Anhyd.Glu) is the molar mass of anhydroglucose (162.1 g/mol). From the DS value, the acyl content (AC) is calculated using the equation below:

$$AC = \frac{DS \times 100\% \times M(\text{acyl group})}{\left(DS \times \left(M(\text{acyl group}) - 1\frac{g}{mol}\right) + M(\text{Anhyd.Glu})\right)} \quad (8)$$

where the molar mass for each acyl group M(acyl) is 43.04 g/mol (acetyl), 57.07 g/mol (propyl), and 71.10 g/mol (butyryl).

FTIR spectroscopy

Secondly, Fourier transform infrared spectroscopy (FTIR) is commonly used to determine the effects of reaction parameters on starch molecular structure, but it can also quantitatively calculate the DS values. The advantage of using FTIR is that the sample measurements are performed in a dry condition without intense sample preparation. However, it is an indirect measuring method for determining the DS. FTIR measurement is used to confirm the DS of esterified starches by determining the absorbance intensity of acyl groups of samples. In this work, the absorbance intensity of the acyl group (their ratios) versus the DS was determined based on the Beer-Lambert law. A previous study from Fei et al., [127] stated that the reliability of the linear relationship of absorbance intensity and DS can be obtained for a DS below 2.0. In this work, SCFA starches were analyzed by a Tensor 37 FTIR-spectrometer (Bruker, Bremen, Germany) with a wavenumber range of 4000–400

cm^{-1} at a resolution of 2 cm^{-1}. The transmittance spectra were baseline corrected and converted into an absorbance spectrum.

Nuclear magnetic resonance (NMR)

Thirdly, NMR was also performed to determine the DS of SCFA starches. NMR is a molecular identification technique that can demonstrate structural evidence by providing information on protons and carbon shift of methyl groups in anhydroglucose units and anhydride groups. NMR is less time-consuming compared to the titration method. However, samples must be dissolved completely in a specific nuclear magnetism reagent to get an accurate result. Thus, this method is considered expensive due to the NMR standard reagent needed. In this work, ^{1}H-NMR was chosen as it can effectively determine the content of acyl groups in starch since the chemical shifts of protons in methyl groups of the acyl groups and anhydroglucose in starch are different. Experiments were performed on an Agilent UNITY Inova (Agilent Technologies, Ltd., Santa Clara, United States) operating at 500 MHz for ^{1}H-NMR. Samples were dissolved in DMSO-d_6 at 85 °C and were analyzed at 45 °C, with 4.8 s relaxation delay and 128 scans. For better quantification, DMSO-d_6 was used to increase the solubility of starch α-dextrins [128]. The DS values were calculated using the following equation [129]:

$$DS = \frac{(A \times 4)}{(3 \times C)} \quad (9)$$

where A is integral of the methyl signals and C is the integral of the proton signals of the anyhydroglucose unit.

Characterization of starch granules

Starch modification affects the physicochemical properties of starch and thus influences its application and function. Such knowledge regarding the physicochemical and structural characteristics of SCFA starches is therefore essential for the rational design of products in food processing, industrial application and physiological function. Through the work, the relationship between physicochemical and functional properties of SCFA starches with respect to different types and levels of modification can be established. SCFA starch characterization was investigated as presented in **Paper II**.

Physicochemical properties of starches

Protein content

The total protein content of starch samples was analyzed by protein analyzer FlashEA® 1112 N elemental analyzer (Thermo Fisher Scientific, Waltham, USA). Aspartic acid was used as a known standard (Nitrogen content: 10.52). The protein content was calculated based on the nitrogen content of the starch samples with a conversion factor of 6.25.

Dry matter

The dry matter of starch was determined according to the AOAC method 2000. The starch sample was weighed out in a sample size of 1 g ± 0.1 and placed in the drying oven for 16 h at 105 °C.

Amylose content

Amylose content was measured according to the 96-well plate iodine binding assay dual-wavelength method developed by Kaufman et al., [130]. The absorbance of each well was quantified at 620 nm and 510 nm using a spectrometer-based absorbance microplate reader (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany). The amylose content was measured by plotting a standard curve obtained by absorbance difference (640 nm and 540 nm).

The amylose content was measured using a dual-wavelength amylose equation as shown below:

$$\text{Dual-wavelength amylose} = \text{Diff ABS} - (\text{y-intercept of regression}) \quad (8)$$

where Diff ABS is the absorbance difference of 620 nm and 510 nm (ABS 620-ABS 510), and y-intercept of regression is the regression obtained from the amylose standard curve.

X-ray diffraction (XRD)

The X-ray pattern of starches was obtained using Small Angle X-Ray Scattering (SAXS) (GANESHA 300XL instrument, SAXSLAB, Copenhagen, Denmark). Diffractograms were acquired at a diffraction angle of 2θ from 5º to 30º at room temperature. The background of the empty sample holder was subtracted with a Kapton window.

Morphology

The morphology of native starch granules was studied using a scanning electron microscope (DELPHI PhenomWorld Delmic, Delft, The Netherlands). The starch samples were mounted on an SEM aluminum holder with double-sided adhesive

tape and were sputtered with 20 nm palladium/gold. The images were captured at an accelerated voltage of 5kV.

Functional properties of starches

Thermal properties

The thermal properties of native and SCFA starches were characterized by differential scanning calorimeter (DSC, Seiko 6200. Seiko Instruments Inc., Japan). Starch dispersions in buffer were prepared at a ratio of 1:5 (w/w). The starch dispersion was homogenized with a rotor-stator high shear mixer (Ystral D-79828) at 22,000 rpm for 60 s and was sealed in an aluminum pan. Starch samples were heated from 10 °C to 120 °C with a scanning rate of 10 °C min^{-1}. The temperature at the onset gelatinization (T_O), peak gelatinization temperature (T_P), final gelatinization temperature (T_C), and gelatinization enthalpy (ΔH) were determined.

Pasting properties

The pasting properties of the native and SCFA starches were analyzed by Rapid Visco Analyzer (RVA) 4800 (Perten Instruments, Perkin Elmer, NSW, Australia). Approximately 25 g of phosphate buffer were weighed out into a plastic canister. Then, 3.5 g of starch sample based on dry weight was added to the canister, and the starch suspension was mixed thoroughly. The measurement was then started at a holding temperature of 50 °C for 1 min, followed by heating to 95 °C for 3.5 min and holding at the same temperature for 2.5 min. The sample was then cooled at 50 °C for 4 min before the final measurement by holding the temperature at 50 °C for 2 min. The peak viscosity, breakdown, setback, final viscosity and pasting temperature were determined.

Texture profiles

The gelatinized starch sample obtained from the RVA analysis was used in this analysis. The gel was allowed to sit for 24 h in the closed canister. The texture profile of solid gel was analyzed by Texture Analyzer (TVT-300XP, Perten Instruments AB, Hägersten, Sweden). Using a 20 mm cylinder, the gel in the canister was punctured at a rate of 1.0 mm s^{-1} to a depth of 10 mm by a double cycle penetration.

Starch hydrolysis

The *in vitro* starch digestion was done following the method by Englyst et al., [131], with slight modification. The reducing sugar was calorimetrically measured by 3,5-dinitrosalicylic acid assay (DNS assay). The amount of readily digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were quantified

using a spectrometer-based absorbance microplate reader at absorbance 540 nm. The fraction of RDS, SDS and RS was calculated according to Simsek et al., [132].

Characterization of starch stabilized Pickering emulsions

Formulations

Pickering emulsions were formulated at five different starch concentrations (50, 100, 200, 400 and 800 mg per mL oil) using the highest DS of SCFA starches. SCFA starch Pickering emulsions were also formulated using SCFA starches at a different level of DS with a starch concentration of 200 mg per mL oil. The amount of medium-chain triglyceride (Miglyol 812) used in this formulation was 10%. For the *in vitro* digestion of SCFA starch Pickering emulsions, native and SCFA starches at the highest DS were used to formulate Pickering emulsions. The amount of starch used was 200 mg per mL oil, with an oil fraction (Φ) of 30% v/v. The preparation technique of Pickering emulsions was standardized for all experiments. Oil-in-water starch Pickering emulsions with 7 mL in total volume were prepared by dispersing starch in phosphate buffer and mixing using a vortex mixer. The corresponding amount of oil was added to the starch dispersion and the mixture was homogenized in glass test tubes using a rotor-stator high shear mixer (Ystral, Germany) with 6 mm dispersing tool at 22000 rpm for 60 s. The properties and stability of starch Pickering emulsions were evaluated and presented in **Paper III**.

Size and morphology

Particle size distribution

The particle size distribution of Pickering emulsions was measured using a laser diffraction particle size analyzer, Mastersizer Hydro 2000 (Malvern Instrument, Malvern, UK) at a pump speed of 2000 rpm with an obscuration range of 10–20%. The average of the droplet sizes was obtained based on $D_{(4,3)}$ (volume-weighted mean diameter), and $D_{(3,2)}$ (surface-weighted mean diameter or Sauter mean diameter) and the mode of $D_{(4,3)}$.

$$D_{(4,3)} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (10)$$

$$D_{(3,2)} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (11)$$

where d_i is the droplet diameter and n_i is the number of droplets with diameter d_i.

Microscopy

In addition to the quantitative measurements using a laser diffraction technique, the morphology of Pickering emulsions was evaluated using optical microscopy (Olympus BX50, Japan). Microscopic observation can be used to compare the quantitative results obtained from the laser diffraction method and give a better understanding of the droplet distribution of the emulsions. The emulsion was diluted in phosphate buffer at a ratio of 1:5, and two drops of the mixture were placed on the microscopic glass slide without a coverslip. The microscopic images were taken using an objective magnification of 20× and 50× and were analyzed using ImageJ (Image processing and analysis in Java software).

Stability

To evaluate the effectiveness of SCFA starch granules as Pickering emulsifier, the relationship between emulsifying capacity and emulsion stability was investigated. Emulsion stability refers to the ability of an emulsion to maintain its characteristics (i.e. micro and macrostructure) for a long period. Stability is therefore a crucial parameter in understanding the behavior of the Pickering emulsion system, which can be measured using various methods such as creaming index, static or multiple light scattering, visual identification, microscopy and size distribution of emulsion droplets at different time intervals.

Stability kinetics towards gravitational separation

The instability mechanisms of Pickering emulsions caused by gravitational separation (creaming/sedimentation) can be analyzed using Static Multiple Light Scattering (S-MLS), Turbiscan Lab Expert (Formulaction Co., Toulouse, France). The stability of Pickering emulsions by S-MLS was determined based on the Turbiscan Stability Index (TSI), which is obtained from the intensity of backscattering (BS) and transmission (TS) profiles, which are correlated to the physical stability of the emulsion system (Figure 9). In this experiment, global TSI (TSI_T) was taken into account to investigate the emulsion droplet migration phenomena caused by sedimentation and/or creaming, as well as possible changes in size caused by coalescence and/or aggregation. The TSI_T value is the measurement of the overall TSI from the bottom to the top of an emulsion phase. The TSI values were calculated using the equation below:

$$TSI = \sum i \frac{\sum_i |scan_i - scan_{i-1}|}{H} \quad (12)$$

which sums up the evolution of TS or BS light at all measured positions, based on a scan-to-scan difference, over the total sample height (H). The TSI integrates all of the variations detected in the samples in terms of size and/or concentration [17]. Therefore, an increase in the TSI value during storage is interpreted as emulsion

droplet migration, leading to local variations of the concentration in the bottom and top of the sample (i.e. sedimentation or creaming processes, respectively), or an increase in mean droplet sizes (caused by coalescence) that can lead to global variations in the middle of the sample. So, it is necessary to analyze TSI_T as well as emulsion macrostructure and microstructure by means of visual inspection and microscopy.

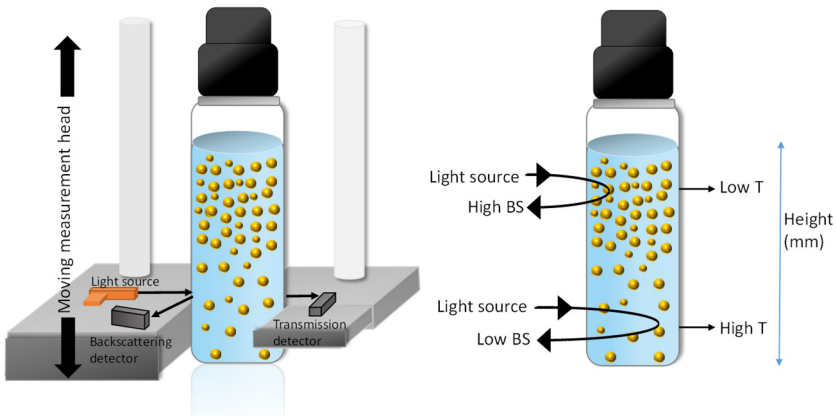

Figure 9. Schematic diagram and parameters of Static Multiple Light Scattering (Turbiscan Lab Expert).

Emulsion Index (EI)

The emulsion index (EI) was identified by measuring the height of the emulsion layer of emulsions by S-MLS. The equation used for the EI determination is stated below:

$$EI\ (\%) = \frac{\text{height of sedimentation or creaming layer (cm)}}{\text{height of total emulsion (cm)}} \times 100 \qquad (13)$$

In vitro digestion of starch stabilized Pickering emulsions

Simulated *in vitro* digestion was performed following three consecutive oral, gastric, and small intestinal phases (Figure 10). All simulated fluids and emulsions were incubated at a temperature of 37 °C before the experiment. An auto-titrator (Titrando 836, Metrohm, Switzerland) including an attached pH probe and dosing unit was used. The digestion experiment was performed in a digestion vessel with a thermostatic jacket that maintained a temperature of 37 °C, and the vessel was connected to the pH-stat that controlled pH under fixed agitation conditions. For the determination of the amount of the free fatty acid released, two references were

prepared as a comparison to SCFA starch stabilized Pickering emulsions. A surfactant-based emulsion, 0.04% w/v of polysorbate 20 in phosphate buffer, and a bulk oil sample were both prepared using the same volume fraction of the dispersed phase (30% of MCT oil) in a phosphate buffer, homogenized using the same emulsification technique as the Pickering emulsions, and carried out using the same *in vitro* digestion model as described for SCFA starch stabilized emulsions. The *in vitro* digestion of SCFA starch Pickering emulsions is presented in **Paper IV**.

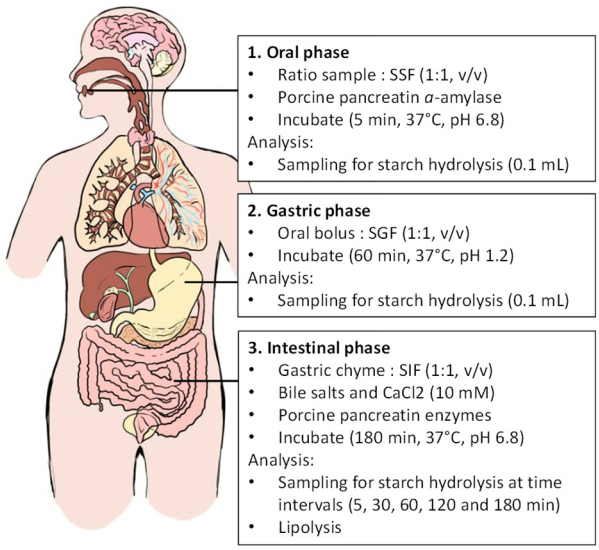

Figure 10. Schematic diagram of parameters used in *in vitro* digestion of SCFA starch Pickering emulsions.

Simulated oral digestion

The simulated oral digestion was performed based on a method by Liang et al., [25] and Mulet-Cabero et al., [108]. The emulsion was mixed with simulated salivary fluid (SSF) at a ratio of 1:1 (v/v). The SSF contained various inorganic salts (12 mM of KCl, 2 mM of KSCN, 7.5 mM of NaH_2PO_4, 4 mM of Na_2SO_4, 5 mM of NaCl, 20.2 mM of $NaHCO_3$ and 0.033 mM of urea). SSF at a volume of 3.8 mL was added to the digestion vessel, followed by 5 mL of the Pickering emulsion and 0.445 mL of uric acid. Once the pH was adjusted to 6.8 and the total volume was adjusted to 9.445 mL, 0.555 mL of the supernatant of the α-amylase solution was gently added to make a total volume of 10 mL in simulated oral digestion. The simulated oral digestion was carried out for 5 min. The material collected at the end of the oral processing stage is called the “bolus”. After 5 min, 0.1 mL of bolus was taken out and analyzed for starch determination.

Simulated gastric digestion

The simulated gastric digestion was performed according to Bai et al., [118], which was essentially adopted from Pharmacopeia [133]. The "bolus" was combined with simulated gastric fluid (SGF) at a ratio of 1:1. The SGF was prepared by mixing 2 g of NaCl with 7 mL of 37% HCl, which was then supplemented with Milli-Q® ultrapure water to reach 1 liter. SGF is typically a highly acidic aqueous solution (pH ≈ 2) containing simulating gastric compositions, such as acids, buffers, salts, and digestive enzymes. However, since native and SCFA starches had a low amount of protein content **(Paper II)**, in this work the pepsin enzyme was not included as it could interfere with the quantification of the hydrolyzed starch (Liang et al., 2016). First, 8.8 mL of SGF was added to the digestion vessel. An allocated amount of 1.2 mL was used to adjust the pH in the simulated gastric system to 1.2, and the amount remaining after the pH adjustment was topped up with the SGF solution so that the final volume of SGF in the mixture was exactly equal to the volume from oral digestion (9.9 mL). The simulated gastric digestion was incubated for 60 min to mimic gastric conditions. The material collected at the end of the gastric stage is referred to as "chyme". At 60 min, 0.1 mL of chyme was analyzed for starch content.

Simulated intestinal digestion

Simulated small intestinal digestion was performed based on a method by Marefati et al., [85] that was adapted from Pharmacopeia [133]. The digestion mixture from the gastric phase was mixed with SIF to reach a ratio of 1:1 (v/v). The simulated intestinal fluid (SIF) consisted of 0.05 M of KH_2PO_4 and 0.2 M of NaOH prepared in a ×1.25 concentration. The corresponding amount of 788 mM of $CaCl_2$ solution, 394 mM of bile salt solution, which was a 50:50 mixture of sodium cholate and sodium deoxycholate were added to set the final concentration of $CaCl_2$ and bile salts in the digestion system to 10 mM. The remaining amount of 1.44 mL was used to adjust the pH to 6.8. The supernatant of pancreatin mixture (1 mL) (10 mg/mL in the final digestion mixture) was added to the digestion system. The simulated intestinal digestion was performed for 180 min and the intestinal materials of this process are called "digest". At allocated time intervals, 0.1 mL of the digest was analyzed for starch content.

Starch hydrolysis

The amount of maltose in 0.1 mL of digestion mixture was quantified by means of a 3,5-dinitrosalicylic acid (DNS) assay based on Lindsay [134] and Teixeira et al., [135] using a spectrometer-based absorbance microplate reader (SPECTROstar Nano, BMG LABTECH GmbH, Germany) at absorbance 540 nm. The amount of maltose was converted to hydrolyzed starch by multiplying by 0.95, which is a conversion factor for starch to maltose [136].

Lipolysis

A pH-stat titration method is a classical approach that is used to monitor the release of free fatty acids (FFA) during simulated *in vitro* digestion. The percentage of FFA released is calculated based on the amount of NaOH required to neutralize two free fatty acids per triacylglycerol molecule generated by lipolysis [109]. To account for the possibility of free fatty acids being released from the SCFA starches, control experiments were carried out where the volume of the oil was replaced with phosphate buffer, and the corresponding type of starch went through the digestion process whereby the amount of NaOH needed to neutralize the drop in pH was recorded. The lipolysis results of SCFA starch Pickering emulsions were subtracted with the corresponding control. In addition, *in vitro* digestion of phosphate buffer mixed with MCT oil (bulk oil) and polysorbate 20 was carried out and lipolysis was measured. The extent of lipolysis from those experiments was then compared to the SCFA starch Pickering emulsions. The amount of FFA% released was calculated using the equation below (Sarkar et al., 2016):

$$\mathrm{FFA\%} = \left(\frac{V_{NaOH} \times M_{NaOH} \times MW_{lipid}}{2 \times W_{lipid}}\right) \times 100 \qquad (14)$$

where V_{NaOH} is the volume (mL) of NaOH consumed, M_{NaOH} denotes the concentration of NaOH (0.5 M) solution required to neutralize the FFA, MW_{lipid} is the molecular weight of the MCT oil (520 g/mol) and W_{lipid} is the mass of the MCT oil initially added to the digestion vessel (1.4175 g).

Summary of the main results

Starch modification and determination of the degree of substitution

Chemical modification and determination of the degree of substitution of rice and quinoa starches were performed as presented in **Paper I**. In this paper, rice and quinoa starches were modified with short-chain fatty acids (acetic, propionic and butyric anhydride). Acetic anhydride has the shortest carbon chain length (C2), followed by propionate (C3) and butyrate (C4) anhydrides. This study aimed to quantify degrees of substitution of SCFA starches using three different techniques.

The DS values were directly determined by means of a stoichiometric equation using the reaction parameters obtained during the esterification (Tables 2 and 3). It was observed that increasing the amount of acyl anhydride increased the amount of acyl group substituted on the starch, thereby increasing the DS values. Depending on their botanical origin, the DS and percentage of acyl obtained for SCFA-rice starches were higher compared to those for SCFA-quinoa starches. The same finding was reported by Colussi et al., [90], who discovered that low-amylose starches are able to undergo better esterification than high-amylose starches, thus increasing the DS. **Paper II** has shown that a low amylose content was found in rice starch (4.20% ± 0.26) compared to quinoa starch (23.16% ± 1.08), which agreed with findings by Marefati et al., [33].

Table 2. Reaction, product parameters and DS obtained from stoichiometry and ^{1}H-NMR analysis of acetylated, propionylated and butyrylated rice starch.

Volume anhydride (mL)	Anhyd (mol)	NaOH (g)	NaOH (mol)	Anhyd.-St. (mol)	Acyl (%)	DS (stoichiometry)	DS (^{1}H-NMR)
Ac-A 1.60	0.01679	28.63	0.01980	0.01378	0.95	0.0360	0.0274 ± 0.0012
Prop-A 2.19	0.01683	31.43	0.02196	0.01170	1.06	0.0305	0.0255 ± 0.0017
But-A 2.84	0.01684	30.74	0.02148	0.01220	1.38	0.0319	0.0314 ± 0.0025
Ac-A 3.20	0.03357	57.88	0.04002	0.02712	1.85	0.0708	0.0642 ± 0.0048
Prop-A 4.37	0.03358	64.03	0.04475	0.02241	2.02	0.0586	0.0527 ± 0.0026
But-A 5.67	0.03362	60.72	0.04243	0.02481	2.76	0.0648	0.0681 ± 0.0010
Ac-A 4.80	0.05036	87.84	0.06074	0.03998	2.70	0.1044	0.0906 ± 0.0030
Prop-A 6.56	0.05042	91.42	0.06389	0.03695	3.29	0.0965	0.0869 ± 0.0039
But-A 8.51	0.05046	91.19	0.06373	0.03718	4.09	0.0971	0.1102 ± 0.0029
Ac-A 6.40	0.06715	118.85	0.08219	0.05211	3.49	0.1361	0.1251 ± 0.0053
Prop-A 8.75	0.06724	122.88	0.08587	0.04865	4.28	0.1270	0.1121 ± 0.0007
But-A 11.34	0.06724	124.74	0.08717	0.04731	5.14	0.1235	0.1267 ± 0.0026

Abbreviations: Ac-A = acetic anhydride, Prop-A = propionic anhydride, But-A = butyric anhydride, n (Anhyd.) = mol of anhydride; n(Anhyd.-St.) = mole anhydride bound on starch.

Table 3. Reaction, product parameters and DS obtained from stoichiometry and ^{1}H-NMR analysis of acetylated, propionylated and butyrylated quinoa starch.

Volume anhydride (mL)	Anhyd (mol)	NaOH (g)	NaOH (mol)	Anhyd.-St. (mol)	Acyl (%)	DS (stoichiometry)	DS (^{1}H-NMR)
Ac-A 1.60	0.01679	30.24	0.02091	0.01267	0.87	0.0331	0.0269 ± 0.0026
Prop-A 2.19	0.01683	31.94	0.02225	0.01141	1.04	0.0298	0.0250 ± 0.0015
But-A 2.84	0.01684	31.90	0.02222	0.01146	1.30	0.0300	0.0331 ± 0.0040
Ac-A 3.20	0.03357	60.72	0.04199	0.02515	1.72	0.0657	0.0562 ± 0.0020
Prop-A 4.37	0.03358	61.40	0.04277	0.02439	2.19	0.0637	0.0579 ± 0.0019
But-A 5.67	0.03362	64.03	0.04461	0.02263	2.53	0.0591	0.0598 ± 0.0053
Ac-A 4.80	0.05036	91.35	0.06317	0.03755	2.54	0.0981	0.0852 ± 0.0019
Prop-A 6.56	0.05042	92.38	0.06436	0.03648	3.25	0.0953	0.0943 ± 0.0002
But-A 8.51	0.05046	96.35	0.06713	0.03379	3.73	0.0883	0.0978 ± 0.0025
Ac-A 6.40	0.06715	120.45	0.08330	0.05100	3.42	0.1333	0.1105 ± 0.0058
Prop-A 8.75	0.06724	126.06	0.08783	0.04665	4.12	0.1219	0.1143 ± 0.0061
But-A 11.34	0.06724	129.36	0.09012	0.04436	4.84	0.1159	0.1229 ± 0.0065

In this study, FTIR was chosen to compare the DS obtained from the stoichiometric equations. FTIR is an indirect measuring method for quantifying DS based on the peak absorbance intensity. Hence, the selection and determination of characteristic peaks and the baseline are important. SCFA starches exhibited a new peak at 1730 cm^{-1} which was assigned to the carbonyl (C=O) vibration. This indicated that the acylation process took place on the starch macromolecule [137, 138]. A positive correlation was observed between the absorbance intensity obtained from the integral (1781–1690 cm^{-1}) and the acyl content obtained from the stoichiometric calculations (Figure 11).

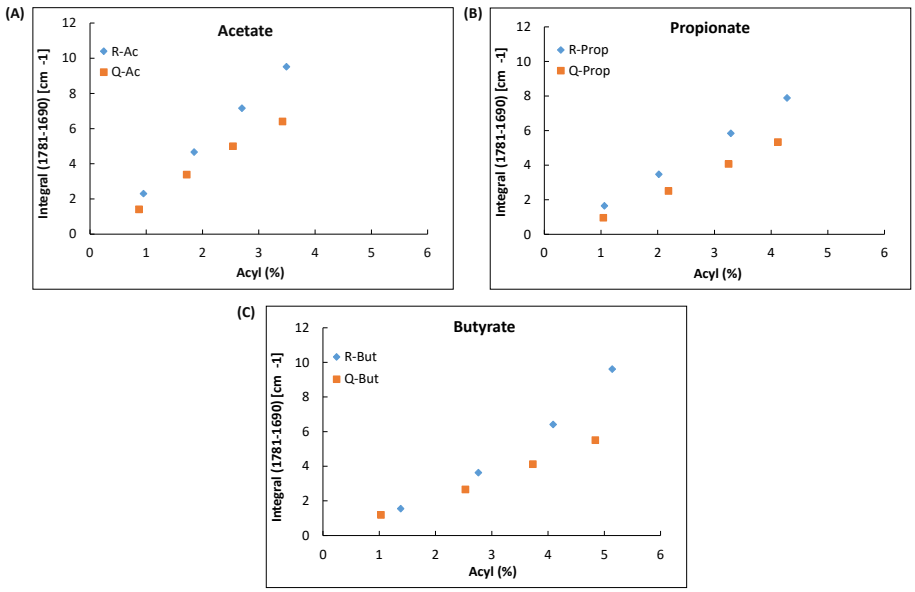

Figure 11. Correlation between acyl content obtained from the stoichiometric calculations and integral values of the carbonyl (C=O) stretching vibration band from FTIR of esterified starches; (A) acetylated rice and quinoa starches, (B) propionylated rice and quinoa starches, and (C) butyrylated rice and quinoa starches.

The ^{1}H-NMR spectrum of acetylated starches displayed a methyl group signal at 2.04 ppm, while the spectrum of propionylated starches presented two new peaks related to the methylene (2.33 ppm) and the methyl group (1.02 ppm) [93]. Butyrylation of starch gives rise to three new peaks observed at 2.27 ppm and 1.55 ppm for the methylene groups and 0.89 ppm for the methyl group (Figure 12). The ^{1}H-NMR spectra exhibited an increased intensity of assigned methyl and methylene peaks as DS increased. The DS values from ^{1}H-NMR appear were within the range with those DS values obtained from the stoichiometric calculations (Table 2 and 3).

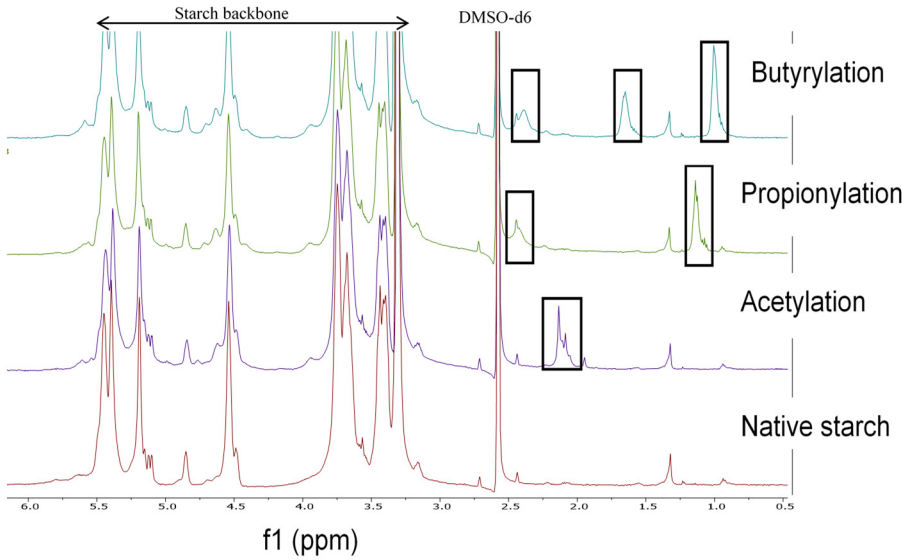

Figure 12. A representative of 500 MHz ^{1}H-NMR spectra of native starch and SCFA starches at different acylation processes.

Overall, **Paper I** described a new direct method that was developed for the quantification of the DS values of SCFA starches using stoichiometric calculations. The DS obtained from stoichiometric calculations were compared with FTIR and ^{1}H-NMR showing positive agreement with both methods. The stoichiometric calculations provide an efficient method for the determination of DS, with no loss of sample and prevention of sampling errors. An important aspect of the application of stoichiometric calculations is that the DS determination is performed simultaneously during the acylation reaction on the entire amount of starch undergoing modification, which not only reduces error, but also makes it possible to control the reaction. The results verified that the DS can be directly calculated using stoichiometric calculations. This method can be used as a predetermined step before further confirmation with FTIR, ^{1}H-NMR, wet titration or other DS determination methods.

Physicochemical and functional properties of starches

Physicochemical properties of starches

The size distribution measured by laser light scattering of native rice granules was observed to have a bimodal distribution, where the mode $D_{(4,3)}$ of the first and the second peak was 1.2 ± 0.0 µm and 5.6 ± 0.0 µm, respectively. Meanwhile, native quinoa starch granules had a unimodal distribution, where the mode $D_{(4,3)}$ was 1.6 ±

0.0 μm. The average diameter size of granules was in the range of 5.0–6.6 μm for SCFA-rice starches and 1.6–1.9 μm for SCFA-quinoa starches. No significant difference was observed in particle sizes before and after esterification ($P > 0.05$).

The images obtained from the scanning electron microscope showed that both native and SCFA-rice starch granules showed the presence of small polyhedral and irregular shape, whereas, native and SCFA-quinoa starch granules displayed a polyhedral shape, with a smoother and more uniform surface. SCFA starch granules maintained their original polyhedral shape before and after chemical modification. It was observed that acetylated and propionylated rice starch granules had slightly rough granular surfaces and somewhat lost their polyhedral shape compared to the native and butyrylated rice starches. This can be attributed to the effect of alkali treatment during the acylation, Surface gelatinization as a result of alkali exposure during esterification may cause the surface of the granules to gelatinize to a minimal extent without losing their granular shape or appearance of fissures [86, 139].

A low amount of protein was measured in the native rice starch (0.283%); while the protein content in native quinoa starch was significantly higher (0.687%). Upon modification, SCFA-rice and SCFA-quinoa starches showed a reduction of protein with the amount of protein in the range of 0.218–0.250% for SCFA-rice starches and 0.537–0.619% for SCFA-quinoa starches. No correlation was observed between the protein content, levels of modification and the type of SCFA group of starches.

Native rice starch was measured to have a low amylose content which was 4.20% ± 0.26 compared to native quinoa starch which amylose content was 23.16% ± 1.08. The SCFA esterification significantly reduced ($P < 0.05$) the amylose content of SCFA-rice starches, while the reduction of amylose in SCFA-quinoa was observed to occur at $DS > 0.06$ (Figure 13). Similar findings addressed that amylose content decrease by esterification as esterification may disturb the amorphous regions of starch granules [140]. The amylose might leach out and be washed away during the washing step after the esterification reaction [140, 141].

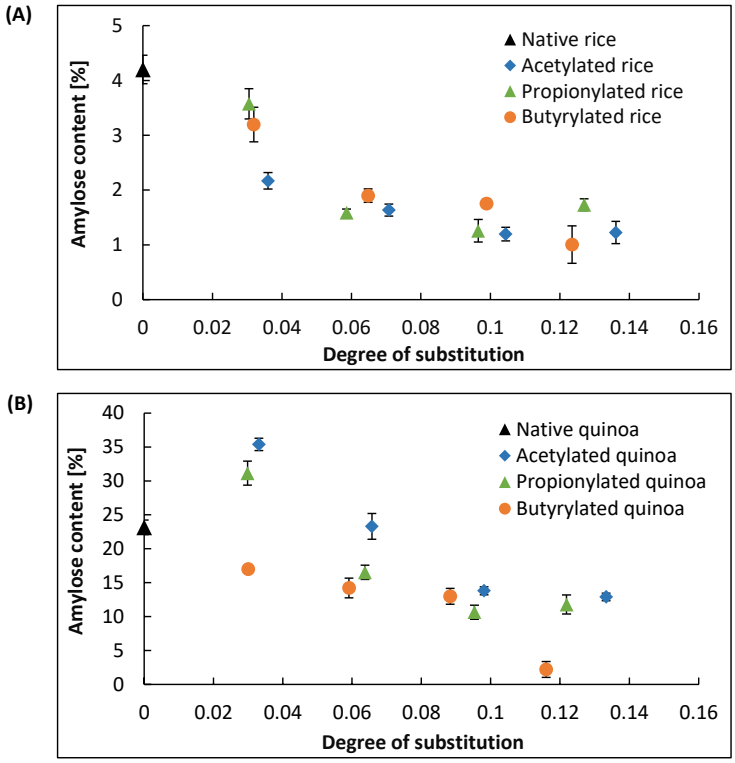

Figure 13. Amylose content of native starches and SCFA starch at different degrees of substituition; (A) rice and (B) quinoa.

The X-ray diffractograms of native and SCFA starches displayed peaks at 15.10°, 20.38° and 22.58° and unresolved peaks at 17.10° and 18.2° (2θ), which explained that native and SCFA starches were an A-type crystalline structure **(Paper II)**. The intensity of the observed peaks corresponds to the crystalline proportion of starch, where the intensity peaks of SCFA starches decreased as levels of modification increased due to the esterification of the acyl group taking place at the hydroxyl sites of the starch structure. It was noticeable that the crystallinity peaks increased at the highest levels of modification of butyrylated rice and propionylated quinoa. At a certain amount, an acyl group can occupy the amylopectin regions, thus creating a new crystalline arrangement in the esterified starch [142].

Functional properties of starches

Structural or composition changes of starch by SCFA esterification may induce changes in the thermal properties of starch. Therefore, functional properties such as thermal properties of starches were investigated. Thermal properties of starch are represented by the temperature of onset (T_O), peak (T_P), conclusion (T_C) of gelatinization and enthalpy (ΔH) (Figure 14). The T_O of native rice was higher than

native quinoa, which agrees with the findings of previous studies [33, 143]. Compared to the native starches, SCFA starches showed lower T_O values, except for acetylated starch at the lowest level of modifications. The T_P of SCFA starches was significantly lower ($P < 0.05$) than their native counterpart. This showed that the SCFA modification altered the thermal properties of the starches compared to their original native forms, even though it was believed that acetylation of starch with the lowest amount of acetic anhydride did not influence the thermal properties of starches.

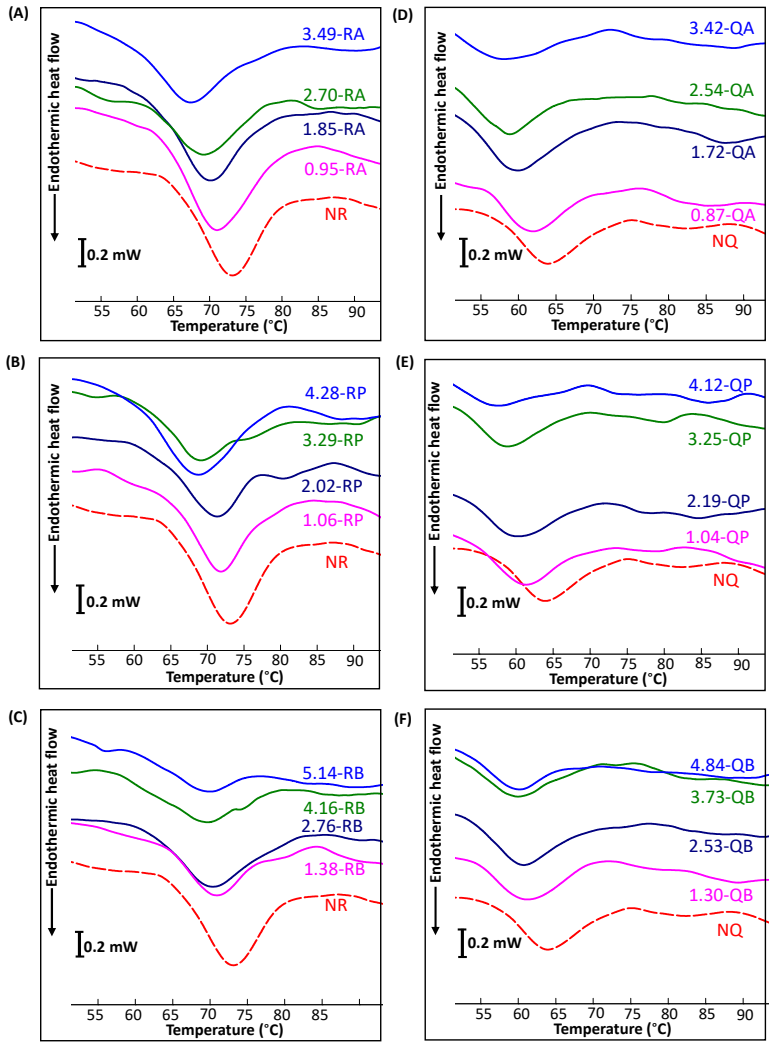

Figure 14. DSC thermogram of native starches and SCFA starch at different types and levels of modification; (A) acetylated rice, (B) propionylated rice, (C) butyrylated rice, (D) acetylated quinoa, (E) propionylated quinoa, and (F) butyrylated quinoa.

To investigate the pasting properties of starch, gelation of starch has to take place in the presence of water and heat. During the gelation process, the soluble part of starch starts to absorb water and swell, thereby creating a linkage that promotes gel formation [144]. Native rice and quinoa starches showed significantly higher pasting temperature (PT) than the modified SCFA starches ($P < 0.05$), which was consistent with gelatinization temperature. Once the starch suspension reached its PT, the viscosity increased as the heating continuously increased and held at 95 °C. The starch paste reached its first maximum viscosity, which is called peak viscosity. A higher peak viscosity was obtained in SCFA starches compared to their corresponding native starches. The increase in peak viscosity was due to the disruption of hydrogen bonds of the starch double helices during acylation, which resulted in the absorption of water by amylose in the amorphous parts and loss of its birefringence [145]. Moreover, rather than in a fixed arrangement (native starch), the introduction of acyl groups creates a new arrangement that contributes to the aggregation of starch in the gel system [146], thus, producing soft gels as indicated in the texture profile (**Paper II**).

The texture profile revealed that SCFA starches had a lower gel hardness, less gumminess and more stickiness compared to their native forms. No significant trend was identified in starches modified with different types of SCFA or at different levels of modification. The weakening of the gel structure was more pronounced in SCFA-quinoa starch, indicating disruption of intramolecular hydrogen bonding that hindered amylose chain interaction. This can result in the formation of fewer junction zones, leading to the formation of a weaker gel [147]. The paste clarity of both SCFA starches was lower at the lower levels of modification compared to the higher levels of modification. This suggests that, at a low amount of acyl, the distribution of acyl groups in starch granules was low and only limited to certain regions of starch granule, thus causing small changes in paste clarity of starch [148].

In vitro digestion of starches

The percentage of RDS in native rice starch was higher than in SCFA-rice starches, except for acetylated rice starch at the modification levels below 1.85 (Figure 15). A high fraction of SDS was obtained in acetylated rice starch compared to propionylated and butyrylated rice starches. At longer SCFA chain length (propionyl and butyryl), SDS fraction was reduced, while RS increased. The decrease of SDS could be explained by the low accessibility of starch molecule to the digestive enzyme resulted from the stereospecific hindrance due to the starch modification [149]. High RS content was identified at the highest level of modification in all types of SCFA starches. This correlates with the decrease of RDS and SDS fractions, which means that the starch was less susceptible to the enzymatic activity during the 120 min of *in vitro* starch digestion due to the shielding effect of the acyl group [150]. The RDS in butyrylated quinoa starch was significantly decreased as the DS increased. A high percentage of SDS was observed in

butyrylated quinoa starch at 1.30–3.73% of acyl content, with a significant decreased in the RDS fraction. This resulted from the higher ability of the pancreatin α-amylase enzyme to break down the SDS fraction of the starch substrate. However, at an acyl content of 4.84%, the SDS fraction was not being digested by pancreatic α-amylase at the same rate and, thus, resulted in an increase in the RS fraction. These results revealed that the modification levels had a larger impact on starch digestibility than SCFA types. Similar findings have been reported by Ye et al., [151] and Dai et al., [92] who mentioned that high DS resulted in a high fraction of RS. There was no correlation between amylose content and starch digestibility, as also reported by Sandhu et al., [152] and Ye et al., [151]. This suggests that the esterification of SCFA groups at different levels of the modification was the main source of reduction of the extent of starch hydrolysis.

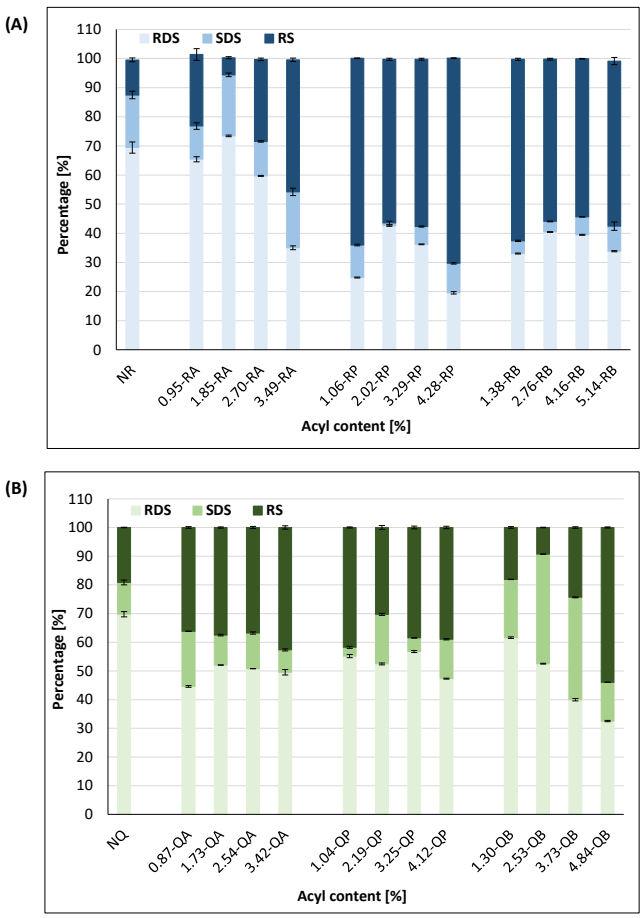

Figure 15. The fraction of RDS, SDS and RS; (A) native rice and SCFA-rice starches and (B) native quinoa and SCFA-quinoa starches.

Principal component analysis

The principal component analysis was carried out to investigate the relationship between starch properties at different types of SCFA and levels of modification (Figure 16). The spread in PC-1 is related to the two different types of starches, which can be observed by the presence of two clusters on the PC-1 axis. Two separated clusters indicated that rice and quinoa starches were not correlated to each other. The PC-2 axis is related to the levels of modification, where the spread along PC-2 varied from native on the positive PC-2 to negative PC-2 for the highest levels of modification. Amylose content did not influence the digestibility of the starches, i.e. SDS, RDS and RS fraction of starches. PCA explained that SCFA types (acetylated, propionylated and butyrylated) did not highly influence starch properties compared to levels of modification.

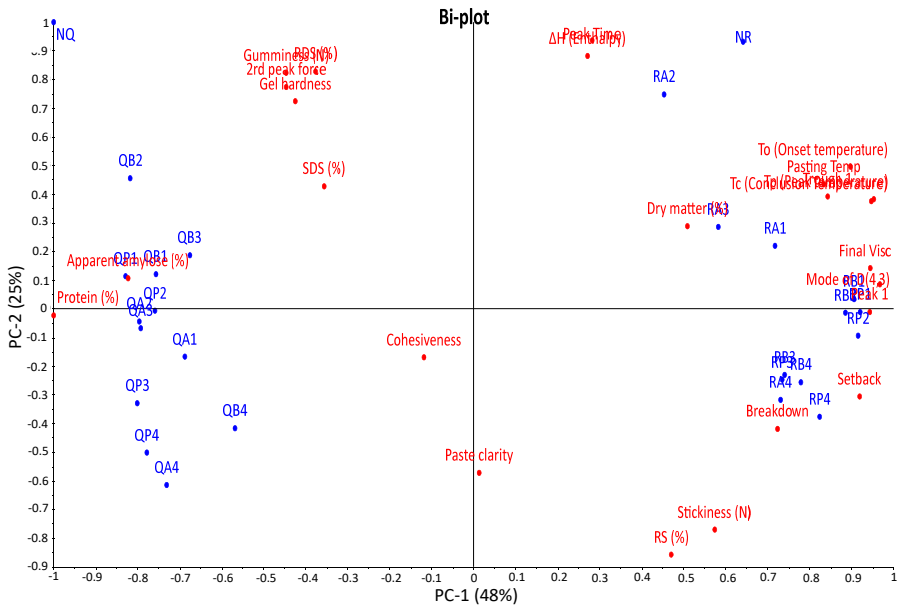

Figure 16. PCA loading plot presented in bi-plot of native and SCFA starches at different types and levels of modification.

In summary, **Paper II** indicated that SCFA starches display various physicochemical and functional properties depending on the types of SCFA and levels of modification. From a nutritional point of view, modification of SCFA-rice and SCFA-quinoa starches at the highest level of modification can result in a high RS fraction. Esterification of starch with SCFA can potentially improve the functional properties of starch as a potential ingredient in food or pharmaceutical applications.

Formulation and stability of SCFA starch Pickering emulsions

Effect of starch concentration on Pickering emulsions droplet size

Size distribution of emulsion droplets is a common measure of the stability of emulsion in a given formulation. At the highest starch concentration, SCFA-quinoa starch Pickering emulsions had the smallest average droplet sizes compared to emulsion stabilized by SCFA-rice starches (Figure 17). The smaller the granule size, the more surface there is to cover at the same starch mass [99]. A higher starch concentration allows better stabilization of the Pickering emulsions. This results from the increased amount of available stabilizer surfaces due to the presence of a sufficient amount of starch particles that can immediately cover the droplet surfaces produced by the homogenizer [26, 33]. Conversely, at the lowest starch concentration, large droplet sizes were observed, attributed to an insufficient amount of starch particles to cover the oil droplet surfaces.

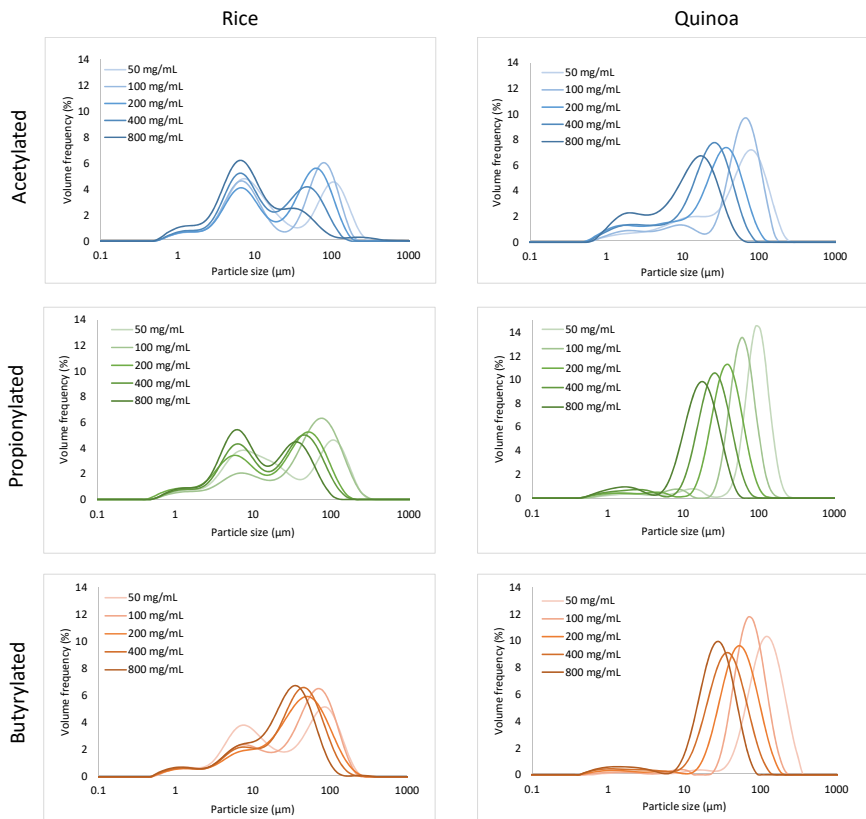

Figure 17. Volume frequency particle size distribution of acetylated, propionylated and butyrylated rice and quinoa starch emulsions at the highest level of modification with 50, 100, 200, 400 and 800 mg starch/mL of oil.

Effect of levels of modification on Pickering emulsion droplet size

In this work, the stability of Pickering emulsions stabilized by native and SCFA starch modified with different levels of modification were investigated. Native quinoa starch had a better emulsifying capacity compared to the native rice starch as was shown by the smaller droplet size distribution of native quinoa Pickering emulsions (NQE) compared to native rice starch Pickering emulsions (NRE), which was represented by the mode $D_{(4,3)}$ of 51.8 ± 0.7 μm and 80.4 ± 7.2 μm, respectively. The first reason for the better emulsifying capacity of quinoa starch was due to the smaller granule sizes that offer larger surface coverage. The second reason was attributed to the higher amount of surface protein, which acts as a natural hydrophobic domain that improves the emulsifying capacity of native quinoa starch. The high protein content in quinoa starch may contribute to a better wettability of starch particles compared to rice starch. Modification of starches with SCFA groups improved the emulsifying capacity, as indicated by the reduction in the droplet sizes and the growth in the volume frequency of the peak representing emulsion droplets as the levels of modification increased. Also, the amount of non-adsorbed starch (free starch) decreased as chain length and level of modification increased, which is another indication of the emulsifying capacity improving (Figure 18). The volume of the emulsion phase was increased in all quinoa-based starch Pickering emulsions that can also be observed by visual observation (Figure 19).

From Figure 18, low levels of acetyl led to the presence of more free starch in the continuous phase. In addition, poor emulsifying properties were indicated by the volume frequency data (Figure 18). This was supported by Hong [153], who stated that the hydrophobicity of starch increases as a result of acylation, which in turn improves emulsifying capacity. It was noted that native quinoa had a better emulsifying capacity than acetylated and propionylated quinoa starches at the lowest level of modification. The protein traces in native starch that can act as natural hydrophobic domains improves emulsifying capacity [33]. The decreased protein content in SCFA starches **(Paper II)** resulted from the removal of protein during the washing steps needed for the esterification.

The mean droplet size of acetylated and propionylated quinoa stabilized emulsions decreased as the levels of modification increased. However, this trend was not observed in butyrylated quinoa emulsions, where the droplet sizes increased as the levels of modification increased. This could be due to the higher extent of droplet aggregation in butyrylated quinoa starch emulsions, as can be seen in micrographs (Figure 21), and the fact that the sizes measured by light scattering were the size of these aggregates rather than a single droplet. Butyrylated quinoa at the lowest level of modification had an emulsifying capacity similar to that of the highest level of acetylated and propionylated quinoa. Thus, to achieve better emulsifying capacity with a low level of modification, increasing the acyl chain length of short-chain fatty acid could be one solution to improve starch adsorption at the oil droplet surfaces. After 50 days of storage, an increase in droplet size (>50 μm) was observed in native

and SCFA-rice starch Pickering emulsions, while the size of most of the quinoa starch Pickering emulsion droplets remained below 50 µm.

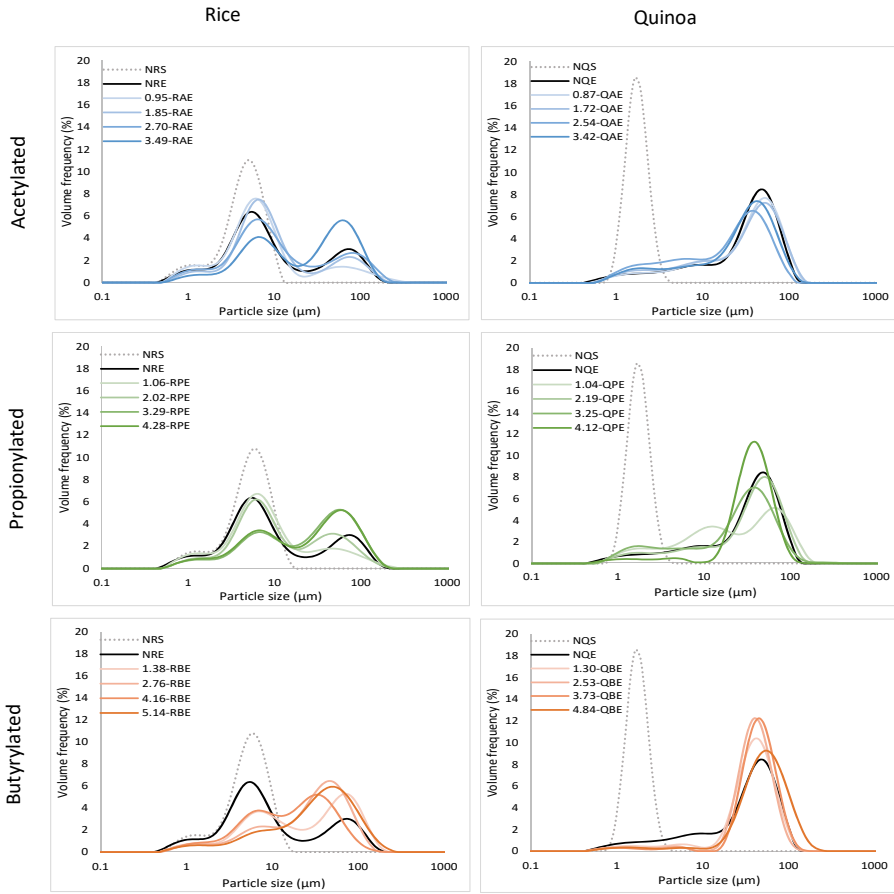

Figure 18. Volume frequency particle size distribution of native rice and quinoa suspensions, native and SCFA starch Pickering emulsions.

Macro-structure of starch Pickering emulsions formulated with native starch and starches with varying levels of SCFA modification

Freshly prepared emulsions showed that native and SCFA-rice starch Pickering emulsions at all levels of modification and chain lengths led to free starch and/or emulsion, which sediments at the bottom of the measuring cells, or a thin layer of emulsion phase or free oil on the top. This observation was observed by visual observation as presented in Figure 19, and was also examined using the S-MLS (Figures 22 and 23). Meanwhile, native and SCFA-quinoa Pickering emulsions

displayed more emulsion phase was formed in all cases with subsequent sedimentation or creaming depending on the resulting emulsion droplet buoyancy (i.e. the relative volume of oil to the amount of starch covering it). Theoretical calculation of emulsion droplet density was performed by the inclusion of the density of starch particles (Figure 21). Theoretically, rice starch stabilized Pickering emulsions tend to sediment due to the higher droplet density, compared to quinoa starch Pickering emulsions at all levels of modification. In this work, it was expected that SCFA-quinoa starch Pickering emulsions would form a cream layer on the top phase due to their small droplets and were believed to have a lower droplet density. However, the cream layer was only present at acyl content above 2.54% acetyl and 3.25% propionyl. The sediment layer in quinoa starch Pickering emulsions was due to the close packing of starch adsorb on the oil droplets (Figure 20). On top of that, the attraction between small emulsion droplets and aggregates might entrap some free starch. This increased the effective density of particle-laden drops of the dispersed phase relative to the continuous phase, hence droplets tended to sink.

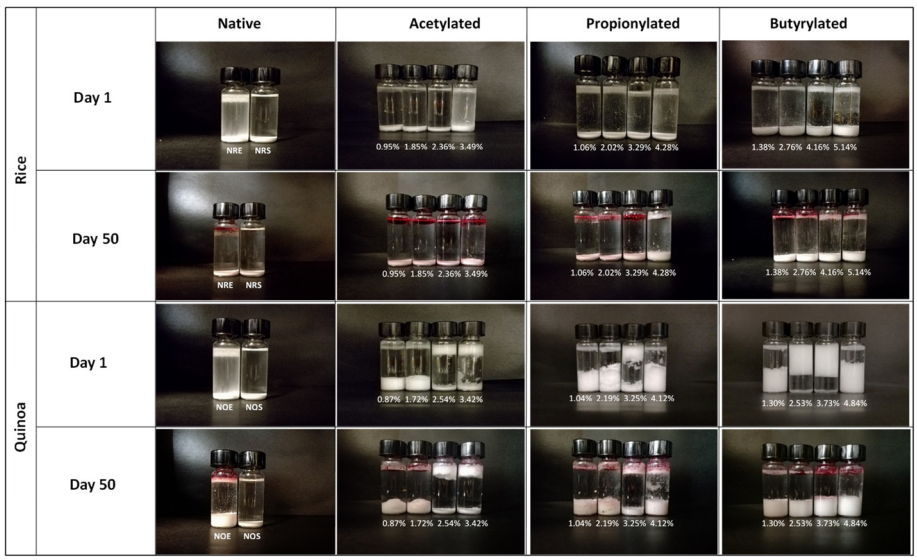

Figure 19. Images of 200 mg starch per mL oil of native starch suspensions, native and SCFA starches on day 1 and day 50. The percentage in the images represents the levels of modification.

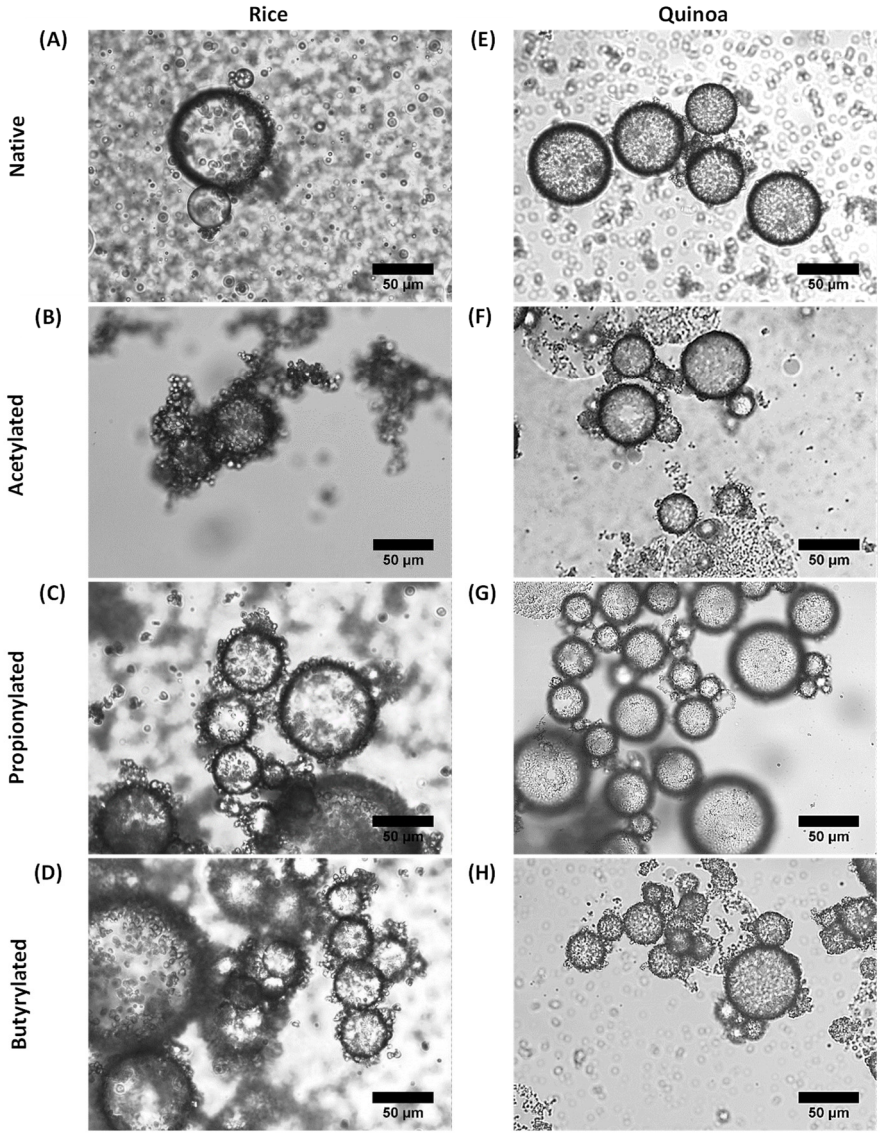

Figure 20. Micrograph images of Pickering emulsions stabilized by native (A: rice, and E: quinoa) and highest modification levels of SCFA starches; acetylated (B: rice, and F: quinoa), propionylated (C: rice, and G: quinoa), and butyrylated (D: rice, and H: quinoa) at formulation of 200 mg starch per mL oil.

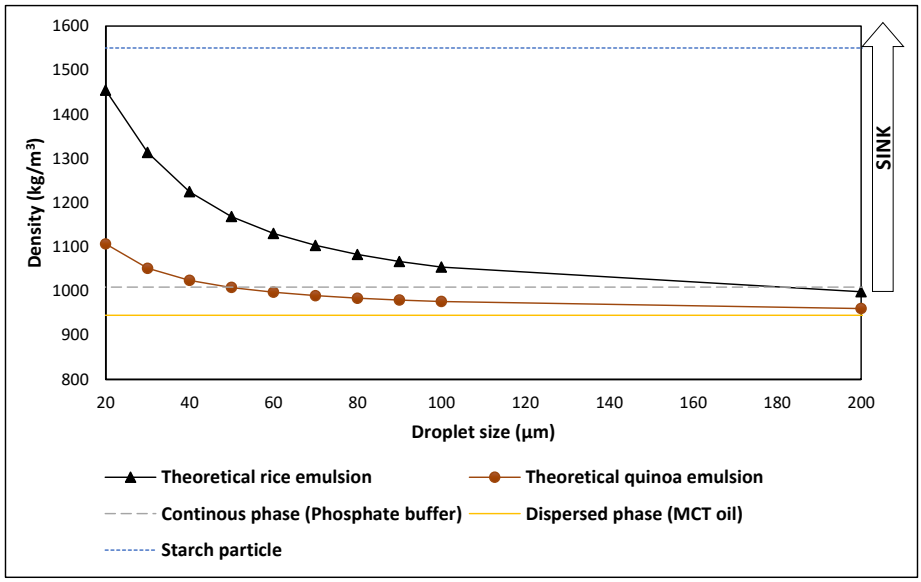

Figure 21. Theoretical emulsion droplet density of rice and quinoa emulsions, if the density is above the continuous phase, the particle or droplet will sink.

Emulsion index (EI) of SCFA starch Pickering emulsions

Emulsion index is a measure of the emulsified layer in an emulsion system which is an indication of emulsion stability [17, 154]. With a range of 58.7–83.0% the SCFA-quinoa starch Pickering emulsions had a higher EI compared to SCFA-rice starch Pickering emulsions, with an EI in a range of 12.0–36.5%. Native rice and quinoa starch emulsions presented the lowest EI, which was 9.5% and 44.4%, respectively. Sedimentation and creaming rapidly occurred in the native and SCFA-rice starch Pickering emulsions. In contrast, droplet migration took more time in quinoa starch Pickering emulsions, owing to the lower density differences between the dispersed and continuous phases (Figure 22 and 23). Quinoa starch Pickering emulsion droplets were smaller than rice starch Pickering emulsions. Hence, there was a smaller density difference between the droplets and the continuous phase in the emulsion system [20].

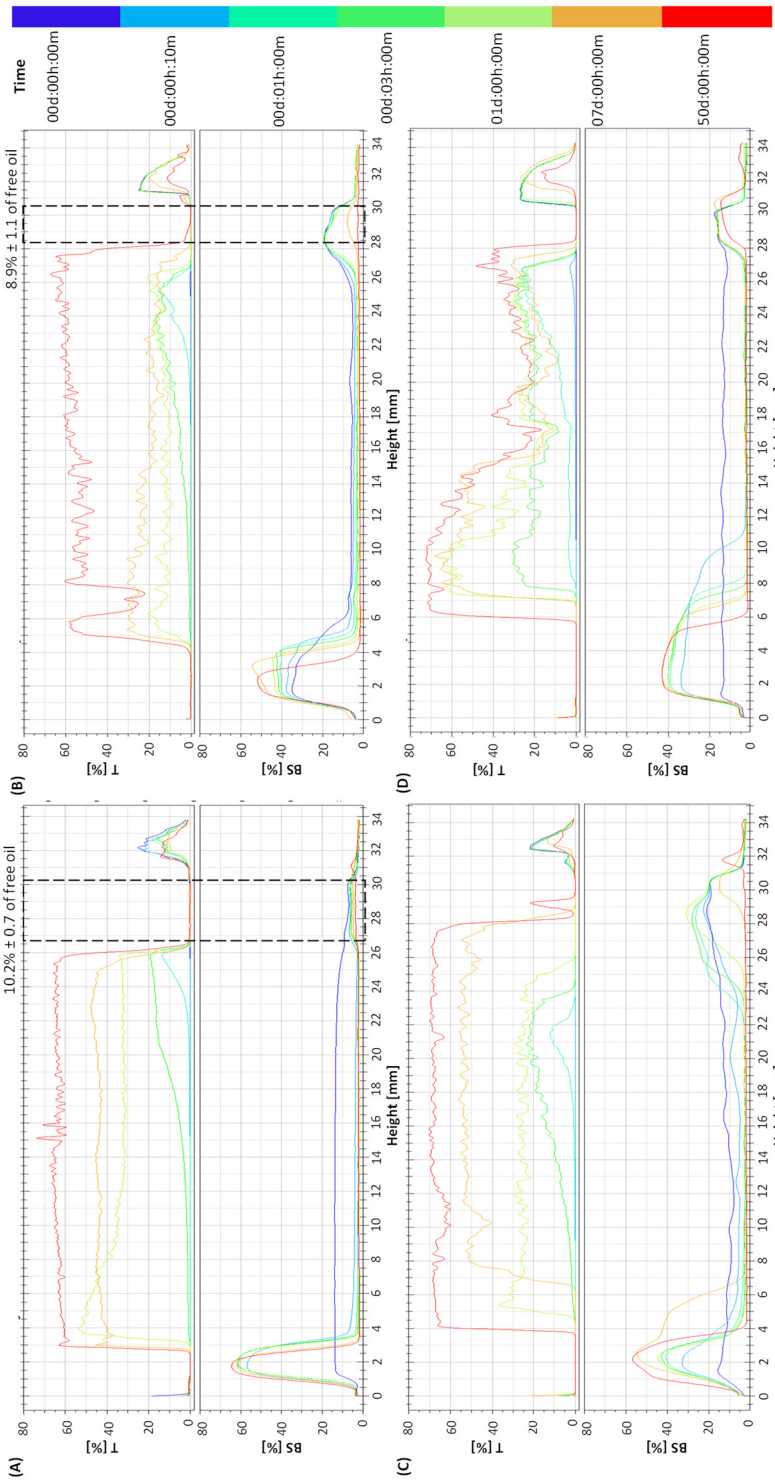

Figure 22. The transmission (T) and backscattering (BS) profiles of Pickering emulsions stabilized by starch granules; (A) native rice, (B) acetylated rice, (c) propionylated rice and (D) butyrylated rice starch at different time intervals. Black dotted rectangular represents the presence of free oil.

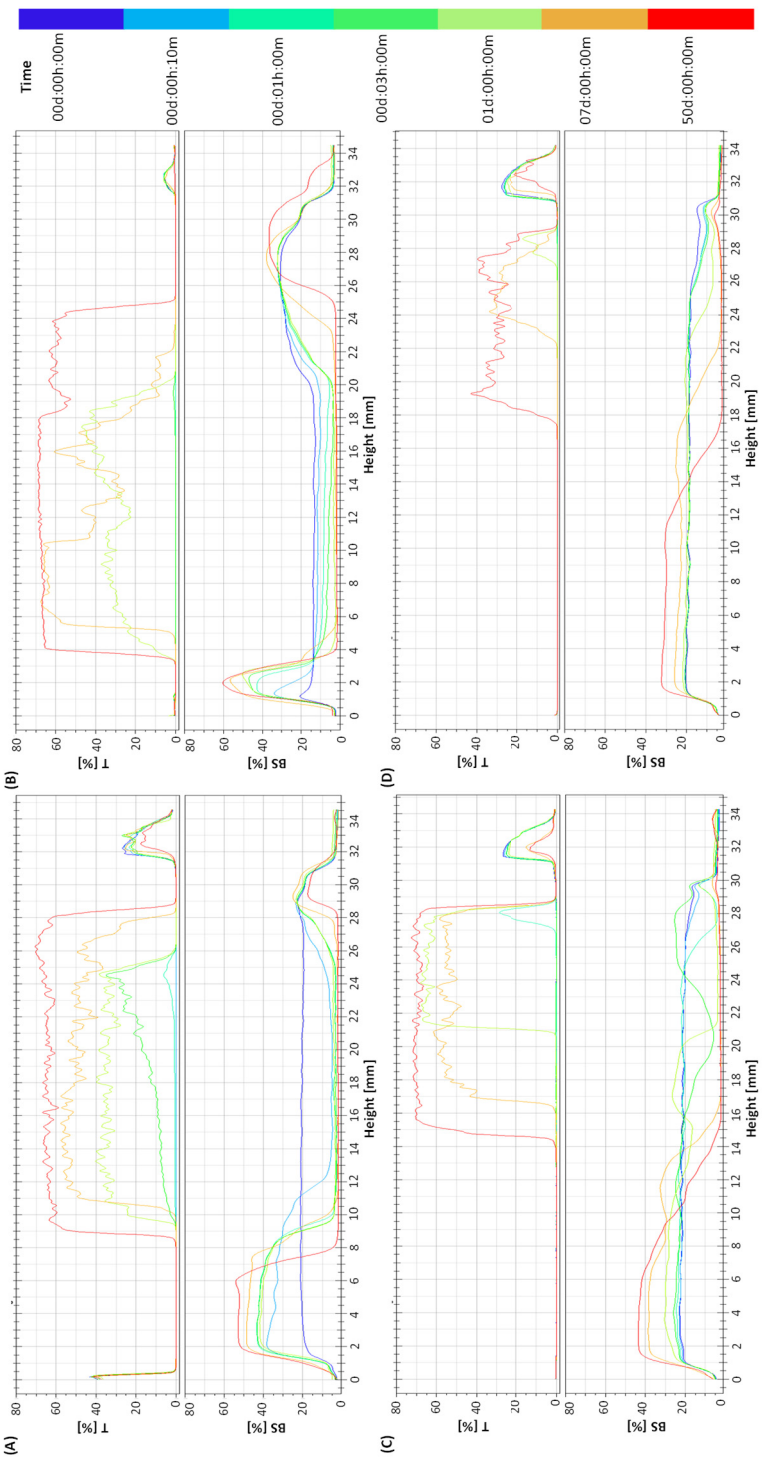

Figure 23. The transmission (T) and backscattering (BS) profiles of Pickering emulsions stabilized by starch granules: (A) native quinoa, (B) acetylated quinoa, (C) propionylated quinoa and (D) butyrylated quinoa starch at different time intervals.

Stability kinetics of SCFA starch Pickering emulsions

The stability of the Pickering emulsion over time was evaluated using the Turbiscan Stability Index (TSI). The TSI integrates all of the variations detected in the samples in terms of size and/or concentration. An increase in the TSI_T value during storage is therefore interpreted as emulsion droplet migration leading to local variations of the concentration in the bottom and top of the sample (sedimentation or creaming processes), or an increase in mean droplet sizes (caused by coalescence) that can lead to global variations in the middle of the sample. A low TSI_T value in SCFA-rice and SCFA-quinoa starch Pickering emulsions means that those emulsions were more stable than their corresponding native forms. Better stability was achieved at higher DS. The longer the chain lengths of SCFA, the lower the BS intensity at the bottom part of SCFA-rice and SCFA-quinoa starch Pickering emulsions. The height of the bottom layer present became wider (Figure 22 and 23). This resulted in the layer of emulsion being less compact, with the presence of droplets and free starch granules. The presence of free starch was noted at the bottom part of the sample for native rice starch emulsions, increasing the BS intensity in that part. Free oil was observed in native and 3.49% acetylated rice starch emulsions after 50 days of storage, 10.2% ± 0.7 and 8.9% ± 1.1, respectively (Figure 22).

In summary, **Paper III** demonstrated that modification in SCFA-rice and SCFA-quinoa starches improved their emulsifying capacity. The substitution SCFA group on starch molecules increased the hydrophobicity and thereby the emulsifying capacity of rice and quinoa starch granules. Increasing starch concentration, level of modification and SCFA acyl chain length resulted in a decrease in droplet sizes, free oil and free starch, as well as better storage stability during the evaluated storage period (50 days).

Simulated *in vitro* digestion of SCFA starch Pickering emulsions

Emulsifying properties of SCFA starch Pickering emulsions

In vitro digestion of Pickering emulsions is an inherently interfacial process as enzymes will be acting on the surface of starch (amylase) and on the interface of oil-water drops (lipase) for hydrolysis to take place. It is therefore important to measure the emulsion droplet size, which can influence the rate of lipolysis. SCFA starches have shown a great emulsifying capacity, as presented by Abdul Hadi et al., [29] (**Paper III)**. Thus, further investigation was carried out in **Paper IV** to investigate *in vitro* digestion of SCFA starch Pickering emulsions with respect to starch hydrolysis and lipolysis. With the formulation of 30% of oil content, native rice and quinoa starch demonstrated droplet size of 53.6 ± 10.1 μm and 36.0 ± 1.0

μm, respectively, which was significantly different ($P < 0.05$) compared to SCFA starch Pickering emulsions. SCFA starch Pickering emulsions exhibited small droplet sizes compared to their native forms. A negative correlation between the SCFA chain length and droplet size was observed, where droplet size decreased as the SCFA chain length increased (NRE > RAE > RPE > RBE) (Figure 24). The mean droplet sizes of SCFA-rice starch Pickering emulsions were 45.2 ± 6.5 μm for acetylated, 41.2 ± 4.6 μm for propionylated, and 36.7 ± 5.5 μm for butyrylated rice starch Pickering emulsions. Quinoa starch stabilized Pickering emulsions gave smaller droplet sizes compared to rice, while a similar trend of decreasing size as the chain length increase was observed (NQE > QAE > QPE > QBE). The droplet size of acetylated, propionylated and butyrylated quinoa starch Pickering emulsions were 29.6 ± 3.7 μm, 31.7 ± 1.3 μm and 31.0 ± 2.4 μm, respectively

Microscopy images showed that SCFA-rice starch particle adsorption on the oil droplets was improved by esterification (Figure 25). Butyrylated rice starch was observed to achieve better droplet surface coverage compared to the native or acetylated and propionylated starches. The SCFA-quinoa starch granules had better surface coverage where starch particles covered the surface of oil droplets to a higher extent. Both native rice and quinoa starch granules were also able to be adsorbed on the oil droplets due to their affinity towards oil, which was attributed to the surface protein of starch granules; however, their performance was not optimal [33].

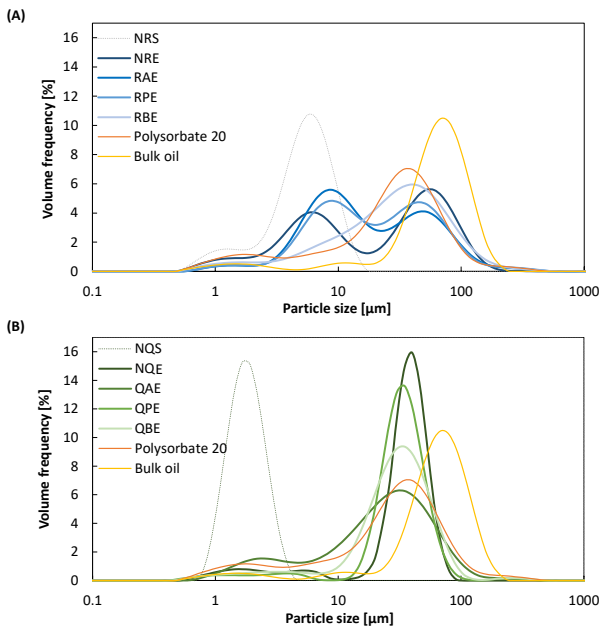

Figure 24. Volume frequency particle size distribution of native starch suspensions, native and SCFA starch stabilized Pickering emulsions; (A) rice starch, (B) quinoa starch, bulk oil and polysorbate 20 stabilized emulsions.

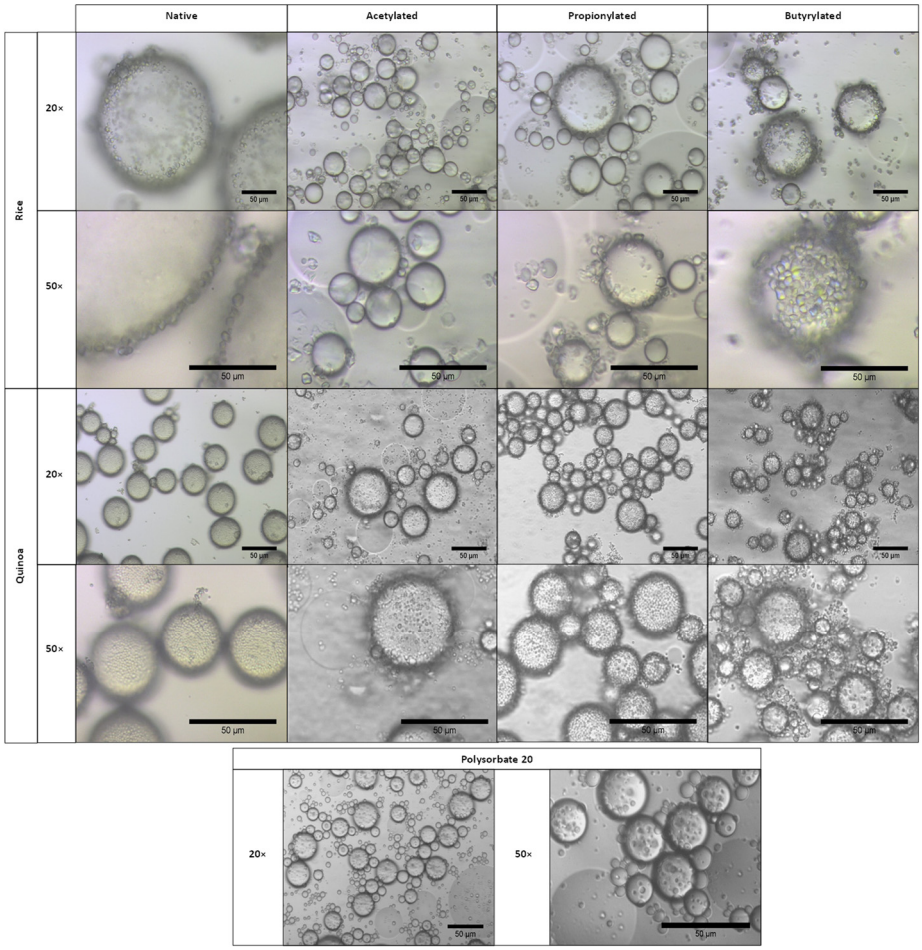

Figure 25. Microscopy images of native, SCFA-rice and SCFA-quinoa starch stabilized Pickering emulsions and polysorbate 20 stabilized emulsions.

Starch hydrolysis

Starch particles are used to stabilize Pickering emulsions by covering the surface of oil droplets. In this case, starch acts as a physical barrier for oil which may first be digested by α-amylases before the lipase reaches the lipids [111]. From this result, the action of α-amylase in the oral phase was not influenced by the type of SCFA starches. No starch hydrolysis occurred during simulated gastric digestion, as the α-amylase was deactivated in the acidic environment of the gastric phase (pH 1.2) (Figure 26). The extent of starch hydrolysis was accelerated by the addition of pancreatic amylase in the small intestinal phase. Native rice and quinoa starches exhibited the highest amount of digested starch, since native starches are easily digestible compared to esterify starches, as was shown in **Paper II**. Furthermore, adsorbed native starches were more easily displaced from the interface of oil

droplets to the bulk aqueous phase compared to esterified starches. In this condition, the displaced starches were free in the aqueous phase, creating more total area available for the action of the pancreatic α-amylase to attack the starch granules' surface. A proposed mechanism is illustrated in Figure 27. Compared to the native starches, butyrylated rice and quinoa starches showed the lowest amount of digested starch within 120 mins of intestinal digestion. Starch modification results in an increase in resistant starch fraction (RS) **(Paper II)**, in addition to increasing the hydrophobicity of starch granules, which delayed the action of α-amylase on the starch substrate [70, 92, 149, 155, 156]. The total digested starch at the end of digestion time for RBE and QBE was 52.3% ± 4.5 and 45.6% ± 4.6, respectively. Butyrylated starch contained more resistant starch, which means that starch digestion is slower within the 120 min of intestinal digestion. Moreover, in this study, it seems that stronger adsorption of butyrylated starch on the surface of the droplets owing to the higher surface hydrophobicity limits the accessibility of pancreatic α-amylase to the site of starch substrate.

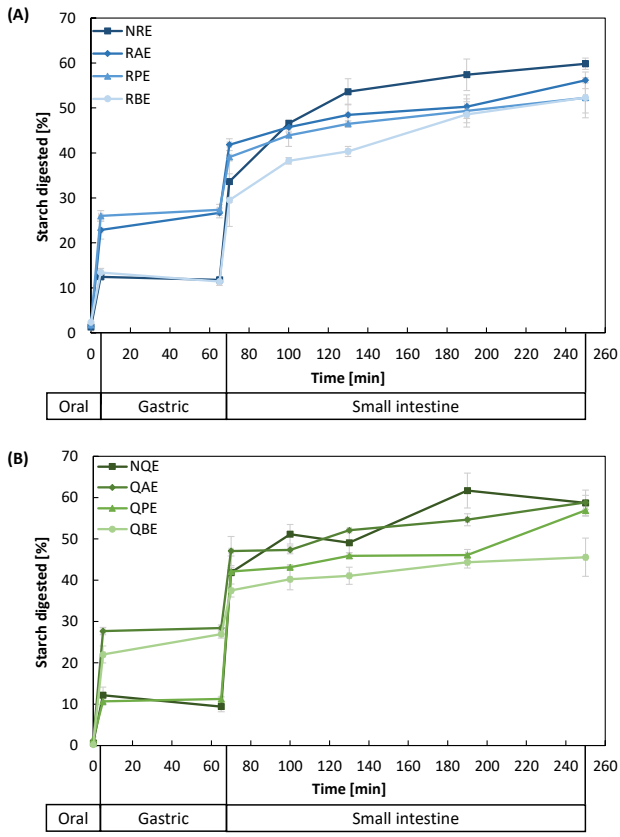

Figure 26. The amount of maltose produced from hydrolysis of native and SCFA starch stabilized Pickering emulsions; (A) native and SCFA-rice starch stabilized Pickering emulsions and (B) native and SCFA-quinoa starch stabilized Pickering emulsions.

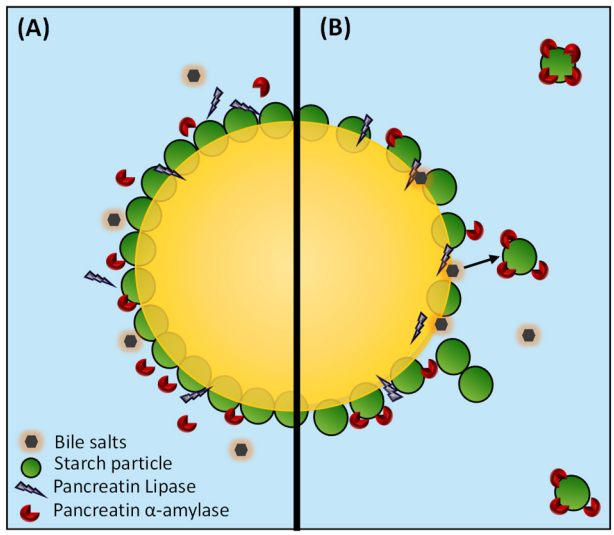

Figure 27. Proposed schematic illustration of starch and lipid *in vitro* digestion of starch stabilized emulsion. Comparison of Pickering emulsions system with (A) complete particles surface coverage and (B) incomplete particles surface coverage.

In vitro digestion of lipid

The adsorption of solid particles like starch to stabilized Pickering emulsions creates interfacial layers with different thickness of surface coverage, which can alter the lipid digestion profile in the small intestine. In addition, lipid digestion is governed by the binding of gastrointestinal components (i.e. bile salts, calcium, or enzyme). Therefore, it is important to investigate the capability of solid particles to protect oil drops from lipid digestion. With such knowledge, lipolysis profiles can be modulated, for example, to reduce the extent of lipolysis induce satiety as well as control delivery of encapsulated bioactive components. In this work, lipolysis was conducted in native and SCFA starch stabilized Pickering emulsions, as well as O/W emulsions stabilized by a surfactant (polysorbate 20), with a mixture of MCT oil and phosphate buffer without the addition of an emulsifier (bulk oil) as control. The control emulsion stabilized by polysorbate 20 was formulated to have similar particle size distribution to that of the Pickering emulsions. In general, all emulsion samples exhibited a relatively similar pattern of lipid digestion, as presented in Figure 28. It was observed that the release of free fatty acids rapidly increased at the initial stage of lipolysis, followed by a gradual increase until it reached a constant value at the end of lipolysis.

During the first 30 min, native and acetylated rice starch Pickering emulsions showed a lower extent of lipid digestion, which was similar to that of the bulk oil (Figure 28). This was due to the poor stability of emulsions with larger droplet size and the presence of free oil, thus lowering the total surface area available for

lipolysis. The initial rate of lipolysis for propionylated and butyrylated rice starch Pickering emulsions was higher attributed to the better emulsifying capacity of their corresponding starches, which created emulsions with smaller average droplet sizes, and thus a larger surface area to volume ratio. This was due to the improved hydrophobicity of these starches, which made them able to have strong particle adsorption on the surface of the oil droplets. Overall, a continuation of the lipolysis revealed that the extent of release of free fatty acids from 1 hour until 3 hours of lipid digestion of native and SCFA-rice Pickering emulsions was not significantly different ($P > 0.05$), with a range of 65–82%. However, native and SCFA-rice starch Pickering emulsions showed a lower extent of lipolysis than the surfactant-based emulsions.

Observation of lipid digestion of the native and SCFA-quinoa stabilized Pickering emulsions had a similar extent of lipolysis during the 10 min of lipolysis (Figure 28). However, the lipid digestion profile started to change after 10 min, where native and acetylated quinoa starch Pickering emulsions had no significant difference until the end of l80 min lipolysis ($P > 0.05$). A lower extent of lipolysis was obtained from propionylated and butyrylated quinoa starch stabilized Pickering emulsions, which were 74.5% ± 3.7 and 58.4% ± 4.2, respectively. Butyrylated quinoa starch Pickering emulsions were able to achieve the lowest extent of lipolysis as a result of higher surface coverage of butyryated quinoa particles surrounding the surface of oil droplets. From the proposed mechanism in Figure 27, the complete surface coverage of particles acts as a physical barrier to prevent the lipase enzyme and bile salts from accessing the lipid droplets. Moreover, strong adsorption of starch particles due to the higher hydrophobicity was shown to prevent, or at least limit, the displacement of starch particles by the bile salts. As a result, the lipase's access to the lipid droplets and other digestion constituents was restricted [118, 157, 158].

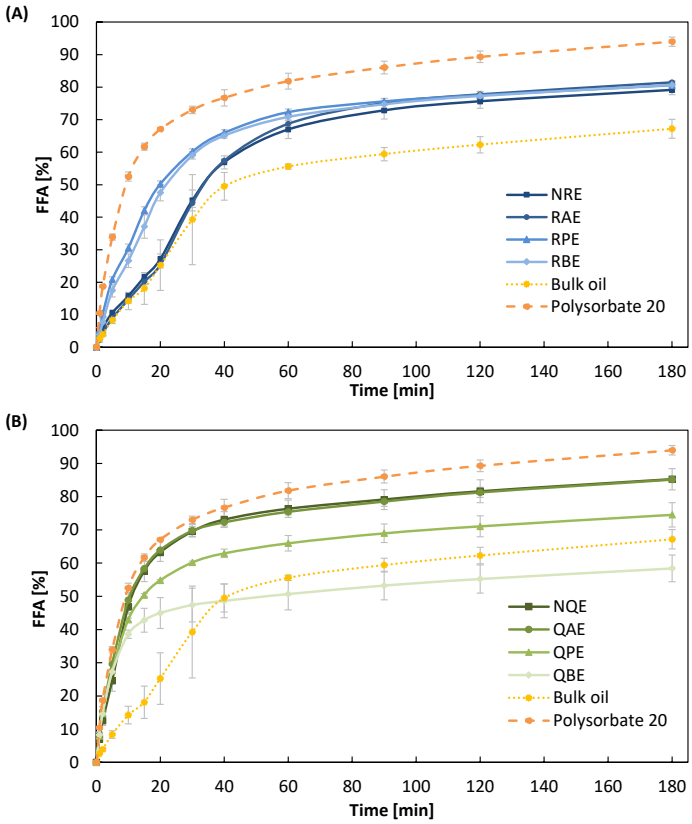

Figure 28. The amount of free fatty acids (FFA) released during simulated small intestinal digestion of starch stabilized Pickering emulsions; (A) native and SCFA-rice starch, (B) native and SCFA-quinoa starch (B), bulk oil and polysorbate 20 stabilized emulsions.

The surfactant-based emulsion has the highest extent of lipolysis (94% ± 1.4) indicating that almost all of the triglycerides in the sample were hydrolyzed. According to Xiao et al., [117], the stabilization mechanism of polysorbate 20 relies on creating a thin layer of the emulsifier at the interface of the oil droplets and is insufficient to protect oil droplets from lipolysis. Furthermore, the small molecular size of polysorbate 20 facilitates its removal by bile salts as a result of small desorption energy compared to starch particles [159, 160]. In contrast, a low extent of lipolysis, 67.2% ± 2.9 was achieved in bulk oil. Even though bulk oil was not protected by any means of stabilizer, its large droplets resulted in the small available surface area for the lipolysis to takes place [124].

The study presented in **Paper IV** summarized that SCFA starches with the longest SCFA chain length displayed the highest stability towards digestion. Esterification of SCFA group on starch molecules hinders starch hydrolysis compared to its native forms. The low extent of hydrolyzed starch in butyrylated starches, particularly for quinoa starch Pickering emulsions, resulted from the high resistance to starch

hydrolysis. Furthermore, a lower amount of free starch in the continuous phase was detected, implying that more starch granules were adsorbed on the oil droplets. In turn, adsorbed starch granules provided less starch substrate site for starch hydrolysis. The extent of lipolysis was retarded by the stronger adsorption and higher packing efficiency of butyrylated starch, which limited the displacement of starch particles from the interface. This, in turn, limited the transport of lipase enzyme to the oil surface for lipid digestion. This study is beneficial for the application of SCFA starches as an emulsifier in the emulsion-based delivery systems, where controlled release and targeted delivery of bioactive compound to the designated site of gastro-intestinal tract is to be obtained.

Conclusions

SCFA-modified starch was successfully prepared via esterification. The presence of new peaks obtained from FTIR and ^{1}H-NMR has shown that the esterification of SCFA groups was successfully achieved on starch molecules. The degree of substitution quantified by a direct stoichiometric method showed similar values to the FTIR and ^{1}H-NMR methods. Thus, the stoichiometric method can be an alternative and faster way to determine the degree of substitution as well as to control the esterification *in situ*.

This work showed SCFA-rice and SCFA-quinoa starches have distinct characteristics compared to their original native form depending on type and level of modification. Amylose, protein content, as well as thermal and pasting properties of SCFA starches decreased by esterification. The textural properties and paste clarity were improved by reducing gel hardness and increasing paste clarity. In terms of functional properties, SCFA starches at the highest level of modification displayed a high fraction of resistant starch. The multivariate principal component analysis provided new perspectives on finding relationships between the different parameters of SCFA starches. The results have shown that the level of modification was more influential on the starch properties than the types of SCFA.

The emulsifying capacity of SCFA starch Pickering emulsions was improved with an increase in the level of modification, the length of the SCFA acyl chain length, and the concentration of starch used in the formulation. Butyrylated and propionylated quinoa starch granules at the highest level of modification produced the smallest emulsion droplet size, higher emulsifying index, no observed presence of free oil, less free starch, and were stable up to 50 days.

In vitro digestion studies of starch stabilized Pickering emulsions showed that SCFA starches were capable of delaying starch hydrolysis and lipolysis compared to native starches and polysorbate 20. Modification of starch increased the resistant starch content in SCFA starches, thereby delaying α-amylase action. Starches modified with the longest SCFA chain length led to stronger starch adsorption on the interface of oil droplets and also created a closely-packed barrier. This may have limited the surface area of adsorbed starch granules exposed to hydrolysis. Additionally, as the hydrophobicity of the starch granules increased by increasing the degree of modification, a higher surface packing was observed. This may have been the reason for the reduction in the rate of lipolysis due to the strong adsorption of starch

particles that were not as readily displaced by bile salts, thereby limiting the access of lipase to the oil surface of the droplets. This work has proven that it is important to modify starch granules such that they can achieve high emulsifying capacity but at the same time have low susceptibility to digestion. This demonstrated that inhibition of starch hydrolysis in the upper digestion stages (oral and stomach) and slow digestion in the small intestine have the potential to deliver a larger fraction of undigested starch to the large intestine for bacterial fermentation. This can both generate SCFA's via the metabolism of the gut microflora and release the SCFAs from the undigested starch particles during bacterial fermentation.

Future perspectives

The application and functionality of SCFA starch Pickering emulsions are two complex subjects that require further investigation. Increasing the depth of understanding regarding the relationship between SCFA starches and their functionality in Pickering emulsions is highly recommended. They are many studies that could be covered in this area as stated below:

1. Starch modification is able to improve the hydrophobicity of starch granules. An investigation should therefore be implemented to locate the site where esterification takes place, whether on the external part of the starch granules (granule surface) or in the internal structure of the starch granules. Advanced instruments such as Spectroscopic PhotoElectron and Low Energy Electron Microscope (SPELEEM) may be capable of detecting the internal composition changes of modified starch.
2. Other than the physical stability of Pickering emulsions, investigation of oxidative stability could be investigated. Oil is one of the main parts of the Pickering emulsion system. Depending on the type of oil used, Pickering emulsions can be subject to lipid oxidation. Understanding lipid oxidation in Pickering emulsions allows researchers to utilize a beneficial oil, such as highly unsaturated fatty acids.
3. SCFA starch Pickering emulsions have shown to reduce digestibility and limit the extent of lipolysis. Further exploration involving encapsulations as well as the release profile of bioactive components or probiotic are topics of interest.
4. Lastly, an *in vivo* study that investigates the gastrointestinal fate of SCFA-modified starch should be considered to determine to what extent SCFA is delivered to the colon via the SCFA starch formulation.

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